P-4

Aloysia gratissima 2n ca. 54

Hymenoxys odorata 2n = 11 II

Phytologia

Contents

Cover Photo. *Aloysia gratissima* and *Hymenoxys odorata* photos by A. Michael Powell. See Powell, Turner and Powell, pp. 96-103.

KEYS TO THE FLORA OF FLORIDA - 24,
CROTALARIA (LEGUMINOSAE)

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ABSTRACT

Crotalaria (Leguminosae) is represented in Florida by 13 species. Of these, 7 are native and 6 are introduced. A reappraisal is provided of the *C. rotundifolia* complex, with five taxa recognized at specific rank. One species, *C. avonensis*, is rated as endangered. Five species reported for Florida are excluded. An amplified key is given to the Florida taxa. *Phytologia 92(1): 3-14 (April 2010)*.

Crotalaria (Leguminosae) is a genus of perhaps 600 species (D. J. Mabberley, 1996), with the great majority native to tropical Africa. In Florida the genus consists of representatives of two sections encompassing five different series. Sect. *Simplicifoliae* is simple leaved, with ser. *Alatae* a difficult complex of native species often marked by inversely sagittate, decurrent stipules; and ser. *Erectae* of robust Old World species. Sect. *Crotalaria* (= sect. *Trifoliolatae*) has ternate leaves, with ser. *Macrostachyae* formed of species largely African in origin, with inflorescences prominent and terminal; ser. *Incanae*, mostly New World tropics, with the style bent downward at right angles to the ovary then abruptly upward; and ser. *Longirostres*, widespread in the tropics, with the style only slightly curved. [This generic structure follows Baker (1914). Polhill (1968) differs in certain respects.]

The native and introduced Florida species are easily assigned: *Crotalaria avonensis*, *C. linaria*, *C. maritima*, *C. purshii*, and *C. rotundifolia* (as well as the native southeastern but non-Florida *C.*

sagittalis) fall into ser. *Alatae*; *C. retusa* and *C. spectabilis* into ser. *Erectae*; *C. lanceolata*, *C. ochroleuca*, *C. pallida*, and *C. zanzibarica* into ser. *Macrostachyae*; *C. incana* into ser. *Incanae*; and *C. pumila* into ser. *Longirostres*.

North American *Crotalaria*, both native and introduced, were well surveyed by H. A. Senn (Rhodora 41: 317-367. 1939), with southeastern species addressed in greater detail by D. Isely (Vasc. Flora S.E. U.S. 1990).   Southeastern unifoliolate species have been monographed by D. R. Windler (Rhodora 76: 151-204. 1974). And the enormous profusion of African *Crotalaria*, a few of which have been introduced into Florida, is excellently treated by R. M. Polhill (*Crotalaria* in Africa and Madagascar. 1982).

The   non-native   *Crotalaria*   pose   little   difficulty   of identification, though their nomenclature is heavily dependent upon taxonomic studies elsewhere, primarily in Africa. Their appearance in Florida is a consequence of the incautious enthusiasm in the early and mid-20th century for plant introductions of foreign species some of which were of great value but others which were either disappointments or actually of major economic and environmental cost. *Crotalaria* has conformed to this pattern. The earliest introduction was of *C. pallida* (then known as *C. striata*) in 1909, soon followed by *C. spectabilis*, *C. ochroleuca* (as *C. intermedia*), and others (R. McKee & C. R. Enlow, U.S.D.A. circ. 137. 1931). Much of the research on these introductions was centered at Gainesville, Florida. By 1931 some 35 species had been grown and tested for agricultural value as green manure and forage, with test samples distributed within the State and throughout the Southeast. Perhaps fortunately, few of these species escaped and have persisted outside of cultivation.   But for those that did escape, a generous literature soon developed addressing their varying levels of toxicity, both green and dried (J. M. Kingsbury, Poisonous Plants of U.S. and Canada. 1964). Quickly, the economic value of the genus was realized to be far less than the potential harm, and commercial use of *Crotalaria* was abandoned. Six species -- five from Africa, one (*C. spectabilis*) from Asia -- remain as an apparently permanent part of the

Florida flora, while a further five species merit listing as having been reported in that status.

The nomenclature of certain of the persisting species has required repeated correction. In several, the name under which they were first introduced has been found to refer to another taxon or to be synonymous of an earlier name (Polhill, 1982). The plant known to McKee & Enlow (1931) and J. K. Small (1933) as *C. striata* DC. (1825), and later by Senn (1939) and R. L. Wilbur (1963) as *C. mucronata* Desv. (1814), has now been assigned a still-earlier name, *C. pallida* Ait. (1789). The plant known to McKee & Enlow and Senn as *C. usaramoensis* Baker f. (1914) has become *C. zanzibarica* Benth. (1843); another name employed for this species, *C. trichotoma* Bojer, has been dismissed as "not a *Crotalaria*" (Polhill, 1982). The plant first known in Florida as *Crotalaria intermedia* Kotschy (1865) and later as *C. brevidens* Benth. (1843) [or *C. brevidens* var. *intermedia* (Kotschy) Polhill] appears to be *C. ochroleuca* G. Don (1832), a second species of a large-flowered narrow-leaflet group of subtly separated African taxa. [The taxonomy of this group is conflicted. Florida materials (FLAS) have been identified as *C. brevidens* var. *brevidens* (R. M. Polhill, pers. comm., Sept. 1982). Their flowers are clear yellow (as in *C. brevidens*), yet their legumes are broad (as in *C. ochroleuca*).]

In contrast, the native species of *Crotalaria*, especially those of series *Alatae* related to *C. rotundifolia*, are nomenclaturally straightforward but taxonomically complex. As treated by Windler (1974), this group consists of 12 species, three native to the American Southeast, the others in Mexico, Central, and northern South America. The southeastern species recognized by Windler were: *C. sagittalis* L.; *C. rotundifolia* (Walt.) Gmel., consisting of var. *rotundifolia* and var. *vulgaris* Windler; and *C. purshii* DC. Windler's study, though answering many questions and relied on by most later workers, did not give fully satisfactory answers as to the number of taxa in the Southeast worthy of recognition nor the characters by which they may best be distinguished.

The present study has concluded that recognition of no less than six *C. rotundifolia*-related species is necessary if the great majority of specimens in the Southeast are to be satisfactorily assigned to discrete groups: (in order of publication) *Crotalaria sagittalis* L. (1753), *C. rotundifolia* Walt. ex Gmel. (1792), *C. purshii* DC. (1825), *C. maritima* Chapm. (1878), *C. linaria* Small (1933), and *C. avonensis* DeLaney & Wunderlin (1989).

Crotalaria sagittalis, the first-named of this complex, though widespread in the continental United States and extending into Mexico, is only an infrequent introduction to the eastern coastal plain. Senn (1939) cited this species from Florida stations in Lake, Levy, and Polk counties, and J. Patman (USF) in an informal study in the 1960s annotated specimens with this name from six additional counties extending well into southern Florida. Not all of these specimens have been examined, but of the several to which access has been possible, only one (Alachua County) appears indubitably *C. sagittalis* -- a cultivated plant from the legume introduction gardens at Gainesville. The other specimens seen have been *C. maritima*, or *C. purshii* with the perennial base of that species not yet well developed, or fragmentary and indeterminate. Windler (1974) and Isely (1990), probably correctly, excluded *C. sagittalis* from Florida, and the species appears not to have contributed to the taxonomic complexity of ser. *Alatae* as seen in the state.

Crotalaria purshii is the coastal plain analogue of the more inland *C. sagittalis*. It is very largely a species of the "high pine," the longleaf pine-wiregrass association once so extensive across northern Florida. By and large, this species is readily recognized by its upright habit and glabrous upper leaf surfaces. But occasional intermediates, apparent hybrids, with *C. maritima* ("*C. rotundifolia*") have been reported by Windler (1974).

Crotalaria rotundifolia is a familiar name but a poorly understood taxon. Though mapped by Windler (1974, as his var. *vulgaris*) as extending south to central peninsular Florida (Hillsborough, Highlands, Polk counties), no specimens have been

confirmed south of a line somewhat farther north in the upper peninsula (Hernando, Lake, Volusia counties). In the northern part of its range, especially in that part north of influence by *C. maritima*, *C. rotundifolia* is recognized by its broadly ovate, often almost orbicular, non-succulent leaves, paler beneath, and pubescent above as well as below; and by stem pubescence in which most hairs are spreading and often longer (2-3 mm.) than the diameter of the stem itself (with an underlay of somewhat appressed shorter hairs).

This species was first published by Thomas Walter (1788) as *Anonymous rotundifolia*, an illegitimate combination (Ward, Rhodora 64: 87-92. 1962; J. Bot. Res. Inst. Texas 1: 407-423. 2007). The name *Crotalaria rotundifolia* was later legitimately published by J. F. Gmelin (1792). But Gmelin's basis was Walter's description and, hence, whatever materials Walter had as a type. Walter, however, kept no types (Ward, Taxon 56: 917-926. 2007). A folio herbarium (BM) prepared in 1787 by John Fraser contains a specimen of *Crotalaria*; it was seen by Walter and labeled by him as "*Lupinus affinis*" ("allied to *Lupinus*"). A series of authors (Fernald & Schubert, 1948; Ward, 1962; Windler, 1974) have termed this Fraser specimen Walter's "type" of *C. rotundifolia*. This designation was corrected to "neotype" (Ward, J. Bot. Res. Inst. Texas 1: 410. 2007).

The Fraser specimen, however, has been repeatedly identified (J. Lewis, N. Robson, D. Windler, D. B. Ward) as corresponding to *Crotalaria maritima*. This taxon, though overlapping the range of *C. rotundifolia* (*s. str.*) is essentially unknown north of southeastern Georgia, to which region Fraser is known to have traveled (and where he may have made the collection). Walter would surely have known the frequent *Crotalaria* of eastern South Carolina and could not have encountered *C. maritima*, and did not recognize the Fraser specimen as the species he had described. I.C.B.N., Art. 9.16 has thus been invoked to supersede the Fraser specimen as neotype of *C. rotundifolia*, and to replace it with a different neotype from South Carolina. This issue has been thoroughly reported elsewhere (Ward, J. Bot. Res. Inst. Texas 3: 219-225. 2009).

Crotalaria maritima was encountered and first described by A. W. Chapman (Bot. Gaz. 3: 4. 1878). Chapman at least once traveled by small boat from Apalachicola, his home in the central panhandle, along the western peninsular coast, to Key West, to visit his friend and correspondent, Dr. John Blodgett, physician and botanist of that city. The date of this journey is unknown, but must have preceded Blodgett's death in 1853. It is likely on this trip, although perhaps on even less documented later trips, that he briefly put ashore on the sandy beach of Cape Sable, the southwestern tip of peninsular Florida. He reported his collection as from "Palm Cape," a largely forgotten name for the central point of the cape. Windler (1974) did not trace this collection site; he reported *C. maritima* only as "Type: not located." A replacement type -- a neotype, in this case also a "topotype" -- has been selected from the Middle Cape of Cape Sable, surely a descendant of the population that Chapman knew so long ago (Ward, J. Bot. Res. Inst. Texas 3: 3: 219-225. 2009).

The distribution of *Crotalaria maritima* in Florida remains uncertain. At times *C. maritima* is dismissed as no more than a synonym of *C. rotundifolia* (e.g., Wunderlin & Hansen, 2003) and as such is stated to occur nearly throughout the state. Though both *C. rotundifolia* and *C. maritima* are found in the Florida panhandle, the *C. maritima* component seems uncommon there; Chapman (1878), it would appear, believed the plant on Cape Sable to be something new and different from plants he encountered near his panhandle home. Northward, it extends into southeastern Georgia, but apparently not beyond.

Crotalaria maritima, as it is found in the southern peninsula, below the southernmost well-defined *C. rotundifolia* in the northern peninsula, is most surely separated by stem pubescence that is loosely upwardly appressed, the individual hairs relatively short, appreciably less than the stem diameter; and by the succulence of the fresh leaves, so turgid they often break when folded, uniformly green above and below. In many populations (as Cape Sable) the leaves of all plants are of similar shape, consistently elliptic to ovate; in other areas leaf shape

will vary on the same plant, the uppermost much narrower, even linear. This charatecter leads into the following taxon.

Crotalaria linaria is allied to and sometimes submerged within *C. maritima* (when that taxon is distinguished from *C. rotundifolia*). J. K. Small first encountered it on Big Pine Key, where it is occasional (G. Avery, his notes, 1963-1967). Later authors have stumbled over this taxon, either reducing it to varietal status (Senn, 1939) or addressing it only as a puzzling footnote (Windler, 1974; Isely, 1990). Certainly *C. linaria* is more closely allied to *C. maritima* than to any other taxon. But the plants are usually small, often upright or nearly so, and at least in the typical form have the diagnostic small linear leaves; the flowers, too, are small (relatively to *C. maritima*), and the calyces are frequently quite maroon-tinted. These morphological distinctions, though often faint or even absent, are to some extent supported by what appears to be a strong habitat preference for coastal dune thickets and dry swales, extending north along both coasts. The taxon is quite absent in the northern range of *C. maritima*.

Crotalaria avonensis is the surprising newcomer to the Florida *Crotalaria* flora. Apparently only a single collection was made prior to the 1980s: Ray Garrett, 22 June 1950, "in deep sand NE of Sebring, Highlands Co., Florida" (FLAS). This specimen was recognized as odd, and was described in some detail: "...a single sheet bearing nine plants or fragments of plants of a *Crotalaria* native to Florida and different from any other species. It is obviously a [*rotundifolia*]-*maritima* derivative, but not a close one. It is from 'deep sand NE of Sebring.' It is a perennial, from deeply buried erect rootstock. Leaves closely crowded on exposed portions of stem, rather uniform in shape on a given plant. The broadest are broadly ovate... Inflorescences are quite erect and extend well above the plant. And, most striking of all, the stems, leaves, and back of the calyx are covered with a dense upwardly-appressed pubescence of coarse stiff straight brown hairs, much in excess of that present on the other species. I'm sure it is one of those scrub endemics of which Small found so many but by no means all." -- [letter to Jacqueline Patman (recently of USF), from D.B.W., 1 Aug 1962]. These observations regrettably were then set aside.

In 1986, quite without knowledge of the Garrett collection, Kris R. DeLaney discovered two populations of the same plant near Avon Park, in the extreme northwestern corner of Highlands County. DeLaney & R. P. Wunderlin (Sida 13: 315-324. 1989) then very fully described and illustrated the new species; it has since been rated as endangered at both State and Federal levels. DeLaney & Wunderlin contrasted plants of the Avon Park populations with nearby (but allopatric) *Crotalaria maritima* ("*C. rotundifolia*" in their interpretation), and found differences in 14 features. Most striking, and to some extent unsettling, was their description and illustration of the style as "smoothly incurved." The other presumably related taxa of series *Alatae* all have abruptly geniculate styles. This character, elsewhere (Polhill, 1968), is treated as of sectional or even subgeneric importance. That it should appear to arise within a Florida population calls for further investigation.

The five species allied to *Crotalaria rotundifolia* and recognized here to hold that rank are unquestionably difficult to treat taxonomically in that an appreciable number of individuals fall into intermediate status. Yet, by treating these five taxa and the non-Florida *C. sagittalis* as of specific rank, it is believed fewer individuals will be seen as aberrant to their assigned species, and the obvious differences they show will not be obscured in a relatively heterogeneous mélange.

The following amplified key reasonably well represents the salient features of the Florida species of *Crotalaria*.

CROTALARIA L.   Rattleboxes [1]

1. Leaves trifoliolate.

    2. Mature legumes <2 cm. long; longest terminal leaflets <2 cm. long; flowers in short several-flowered terminal or lateral racemes. Perennial herb, to 1 m. Open pinelands, dry woodlands, dune swales. South peninsula; frequent on east coast (Dade, n. to Volusia County), rare on west coast (Monroe, n. to Sarasota). All year.   **Crotalaria pumila** Ortega

    2. Mature legumes 2.5-5.0 cm. long; longest terminal leaflets >2 cm. long (or if shorter, plant long-pubescent).

       3. Leaflets linear to narrowly lanceolate.

          4. Flowers 0.8-1.0 cm. long; legumes 2.5-3.0 cm. long, 0.4-0.6 cm. broad. Annual herb, to 1.2 m. Ditches, dry woodlands. Throughout; frequent (rare in panhandle). Summer-fall.   * **Crotalaria lanceolata** E. Mey.

          4. Flowers 1.5-2.0 cm. long; legumes 3.5-5.0 cm. long, 1.5-1.8 cm. broad. Annual herb, to 1.2 m. Roadsides, old fields. Scattered in panhandle (Escambia, Leon, Jefferson counties), south to mid-peninsula (Hardee, Highlands); infrequent. Summer-fall. [*Crotalaria brevidens*, misapplied, *Crotalaria intermedia* Kotschy]   * **Crotalaria ochroleuca** G. Don

    3. Leaflets broadly elliptic to obovate.

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[1] This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

5. Stem, petioles, and legumes loosely covered with long brown pubescence; racemes few-flowered, often overtopped by uppermost leaves. Annual herb, to 1.5 m. Roadsides, sandy fields, beach ridges. South and central peninsula (n. to Hillsborough, Brevard counties); frequent. Spring-summer-fall.   **Crotalaria incana** L.

5. Stem, petioles, and legumes nearly glabrous; racemes many-flowered, extending well above uppermost leaves.

   6. Keel with dark striations; leaflets obtuse to emarginate. Annual herb, to 1.5 m. Roadsides, fencerows, old fields. Throughout; common (rare in peninsula). Summer-fall. [*Crotalaria mucronata*, misapplied; *Crotalaria striata*, misapplied]   * **Crotalaria pallida** Ait. var. **obovata** (G. Don) Polhill

   6. Keel not striate; leaflets acute to obtuse. Annual herb, to 1.2 m. Disturbed areas. South peninsula (Dade County); rare. Summer-fall. [*Crotalaria trichotoma*, misapplied; *Crotalaria usaramoensis* Bak. f.]
                         * **Crotalaria zanzibarica** Benth.

1. Leaves unifoliolate.

  7. Annual; flowers large, ±2 cm. long, upper petal conspicuously longer than the calyx lobes; larger leaves 6-14 cm. long, obovate with broadly rounded apex.

    8. Flowers subtended by minute linear bracts. Erect annual herb, to 0.8 m. Old fields, roadsides. Peninsula; infrequent on lower east coast (Dade to St. Lucie County), rare northward (Polk, Alachua, Leon). Summer-fall.   * **Crotalaria retusa** L.

    8. Flowers subtended by broad foliaceous bracts. Erect annual herb, to 1.2 m. Old fields, roadsides, disturbed areas. Throughout; common. Summer-fall. [*Crotalaria Retzii* Hitchc.] YELLOW RATTLEBOX.
                         * **Crotalaria spectabilis** Roth

  7. Perennial; flowers small, ±1 cm. long, with upper petal scarcely exceeding the calyx lobes; leaves ranging from short (1.5 cm.) and orbicular to long (10 cm.) and lanceolate or linear, the longer leaves always with acute apices.

9. Leaves glabrous above, linear to lanceolate (or lowermost ovate); plants erect. Perennial herb, to 40 cm. Sandy fields, open pinelands. Panhandle and north Florida, south to upper peninsula (Marion, Lake counties); frequent. Spring-summer.
Crotalaria purshii DC.

9. Leaves appressed-strigose above, ovate to elliptic; plants erect to decumbent or prostrate.

  10. Leaves uniformly linear (entire plant), ±2 mm/ wide, 10-15 mm. long; sepals reddish abaxially. Much-branched perennial herb, to 30 cm. Coastal dunes, dry pinelands. South peninsula; keys (Monroe County: Big Pine Key), north along coasts (to Pinellas County on west, Palm Beach County on east); infrequent, rarely adventive inland. Winter-spring. Endemic. [*Crotalaria maritima* Chapm. var. *linaria* (Small) Senn] **Crotalaria linaria** Small

  10. Leaves elliptic to ovate or orbicular (or if some linear, only uppermost on stem); sepals wholly green.

    11. Pubescence of stem spreading or ascending (hair length ± equal to diameter of stem); plants prostrate to decumbent; leaves broadly ovate to orbicular, relatively thin (not breaking when folded), whitish-green beneath, semimucronate (mucron largely formed of clustered hairs). Perennial herb. Open pinelands. Panhandle and north Florida, south to mid-peninsula (Hernando, Lake, Volusia); common. Spring-summer. [*Crotalaria angulata*, misapplied]
RABBIT-BELLS.
Crotalaria rotundifolia Walt. ex Gmel.

    11. Pubescence of stem upwardly appressed (hair length much less than diameter of stem); plants prostrate to erect; leaves thick, succulent (fresh leaves breaking when folded), uniformly green on both surfaces.

      12. Stems prostrate from central rootstock; leaves lance-ovate (or a few linear). Perennial herb, branches trailing to 0.6 m. Dry sands. Throughout; common. Winter-spring-summer. Near-endemic. [*Crotalaria rotundifolia*, misapplied] **Crotalaria maritima** Chapm.

12. Stems erect from buried horizontal rootstock (buried branches?). Perennial herb, to 12 cm. White sand of sand pine scrub. Central peninsula (Highlands County: Avon Park, Sebring); rare (2 known populations). Spring-summer. Endemic. ENDANGERED (Federal, State listings).

Crotalaria avonensis DeLaney & Wunderlin

Excluded names:

Crotalaria angulata Mill.
This name applied in error to the native *Crotalaria rotundifolia* (Senn, 1939; Wilbur, 1963; etc.). True *C. angulata* is Asian.

Crotalaria grantiana Harvey
Crotalaria virgulata Klotzsch
ssp. *grantiana* (Harvey) Polhill
First grown in Florida in 1928 (Renfrow - FLAS; McKee & Enrow, 1931), but not persisting. Only known non-cultivated spm.: "road to Highlands Hammock State Park," Highlands Co. (Garrett, Oct 1948 - FLAS).

Crotalaria juncea L. Sunn Hemp
Sometimes grown as a cover crop or as green manure (McKee & Enrow, 1931), but does not persist outside of cultivation.

Crotalaria sagittalis L.
Reported for Florida (Small, 1933; Senn, 1939; etc.). But not a coastal plain species (Windler, 1974). Spms. examined have been *C. maritima*, *C. purshii*, or *C. rotundifolia*.

Crotalaria verrucosa L.
Noted in 1974 as naturalized at Subtropical Research Station, Dade Co. (Avery 1490 - FLAS). Not otherwise known outside of cultivation.

TWO NEW SPECIES OF *BRICKELLIA* (ASTERACEAE: EUPATORIEAE) FROM OAXACA, MEXICO

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ABSTRACT

Two novelties in the genus *Brickellia* from Oaxaca, Mexico, are described: **Brickellia coixtlahuaca, sp. nov.** and **Brickellia huahuapana, sp. nov.** Both taxa belong to the Barroetea group of *Brickellia,* as envisioned by Turner et al. (1991, 1997). Photographs of the types are provided, along with maps showing their distribution. *Phytologia 92(1): 15- 19 (April, 2010).*

KEY WORDS*: Brickellia, Barroetea*, Asteraceae, Eupatorieae, Mexico, Oaxaca.

BRICKELLIA COIXTLAHUACA B.L. Turner, **sp. nov. Fig. 1, 2**

Brickelliae pavonii (A. Gray) B.L. Turner similis sed differt foliis valde petiolatis (vs sessilibus), pedunculis ultimis brevioribus (2-6 mm longis vs 10-30 mm), et acheniis adaxaliter 2-nervatis (vs 3-4-nervatis).

Delicate, stiffly erect, annuals 15-30 cm high. **Mid-stems** sparsely puberulent. Leaves mostly 2.5-4.0 cm long, 2.0-2.5 cm wide; petioles 3-15 mm long; blades broadly ovate to subcordate, palmately 3-nervate from the base, glabrous or nearly so, the margins serrulate. **Capitulescence** a terminal, somewhat one-sided, corymb, the ultimate peduncles 2-6 mm long. **Heads** ca 8 mm high, each with ca 10 florets; involucral bracts linear-lanceolate, 3-4 seriate, glabrous. **Corollas** 4-5 mm long, 5-lobed, glabrous. **Achenes** flattened, ca 4 m long, the adaxial side with 2 ribs; pappus of ca 20 slender bristles ca 5 mm long.

TYPE: **MEXICO. OAXACA: DISTRITO COIXTLHUACA.** Concepcion Buena Vista, "Km 101.5 de la Tehuacan-Oaxaca (cuota)...Chaparral-selva baja caducifolia. Ocasional." 1400 m, 18 04 30 N, 97 21 09.1 W, 30 Sep 1995, *Jose L. Panero 6145* [with I. Calzada & C.C. Clevinger] (Holotype: TEX).

As noted in the above diagnosis, very similar to *B. pavonii*, and so identified by its collectors.

The species is named for the Distrito Coixtlahuaca, whence the type (Fig. 3).

BRICKELLIA HUAHUAPANA B.L. Turner, **sp. nov. Fig. 1, 3**

Brickelliae laxiflorae (Brandegee) B.L. Turner similis sed differt foliis sessilibus (vs valde petiolatis) et acheniorum paginis adaxialibus valde 4-5-nervatis (vs 1-3 nervatis).

Stiffly erect annual (?) herbs 40-80 cm high.   **Mid-stems** sparingly pubescent with upturned white hairs.   **Leaves** 2.5-3.5 cm long, 1.5-2.5 cm wide; sessile or nearly so; blades ovate, sparingly pubescent above and below, the margins irregularly serrate.   **Capitulescence** a terminal corymbose panicle 20-30 cm high, 15-20 cm across, the ultimate peduncles mostly 2-3 cm long.   **Heads** 10-11 mm high, 10-20 mm wide.   **Involucres** 2-3 seriate, the outermost bracts glabrous, loose and filiform, as long as the inner bracts.   **Florets** ca 20 per head; corollas greenish-white, glabrous, 5-7 mm long, the 5 lobes ca 0.5 mm long.   **Achenes** flattened with 9 well-developed ribs, black, sparingly pubescent; pappus of ca 20 slender,white, bristles 5-6 mm long.

TYPE: **MEXICO. OAXACA: DISTRITO HUAHUAPAN. Mpio. Santo Domingo Tonala,** " paraje yucununi parte baja, Selva seca. orillo de arroyo." ca 1405 m, 17 38 31.5 N, 97 58 54.1 W, 13 Nov 2008, *Luis Angel Hernandez Perez 705* (Holotype: TEX).

ADDITIONAL SPECIMEN EXAMINED: Essentially same locality as above: "Guamil. Sobre cerro." ca 1381 m, 8 Nov 2008, *Hernandez 447* (TEX).

Brickellia huahuapana is a very distinctive member of the Barrotea grouping, what with its robust habit and sessile leaves; in habit it much-resembles *Brickellia subuligera*, but its markedly ribbed achenes rule out such an association.

The species name derives from Distrito Huahuapan, whence the type (Fig. 1).

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Fig. 1. Distribution of *Brickellia coixtlahuaca* and *B. huahuapana*.

Fig. 2. *Brickellia coixtlahuaca* (Holotype).
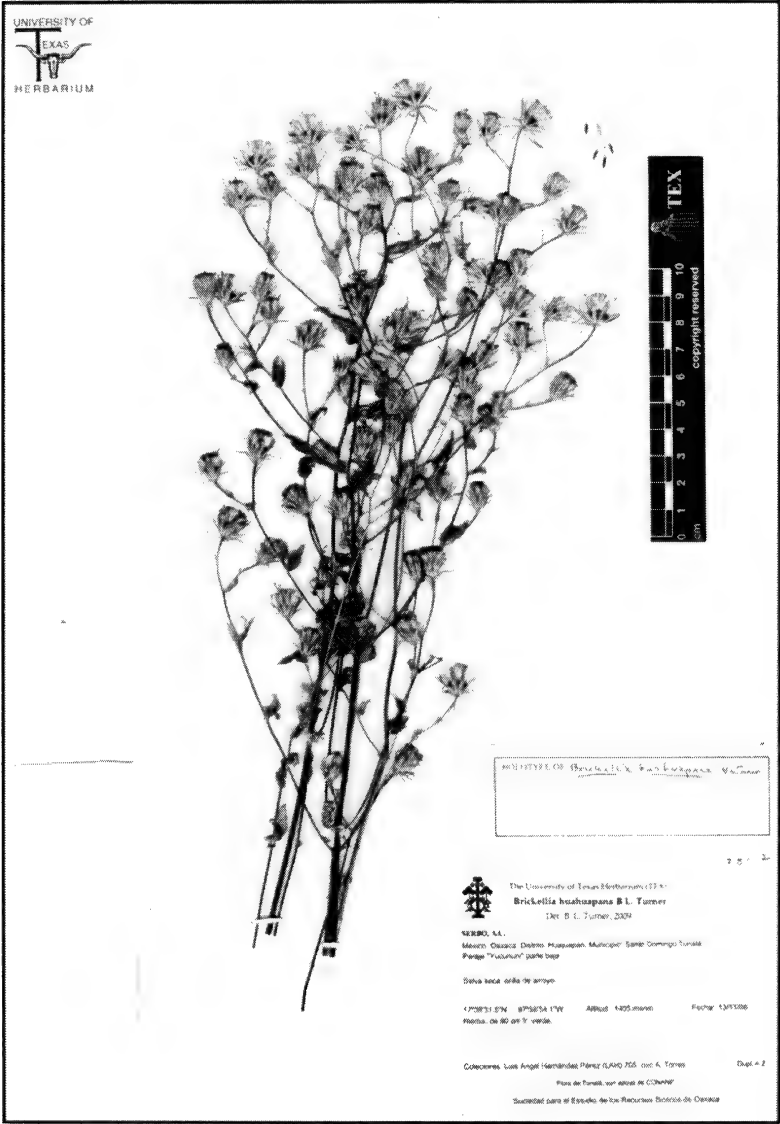
Fig. 3. *Brickellia huahuapana* (Holotype).

RECENSION OF THE MEXICAN SPECIES OF *SALVIA* (LAMIACEAE), SECT. PENINSULARIS

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ABSTRACT

Epling (1939) established the sect. Peninsularis containing two taxa, *Salvia peninsularis* and *S. macei*, both occurring in Baja California Sur. Subsequently (Epling and Jativa, 1968), *S. macei* was reduced to synonymy under *S. peninsularis*. I have added an additional two species to the section, **Salvia lapazana, sp. nov,** and **Salvia moranii, sp. nov.,** both from southern-most Baja California. Photographs of the types are provided, along with a map showing the distribution of the several taxa now recognized in the group. *Phytologia 92(1): 20 - 26 (April, 2010).*

KEY WORDS: *Salvia*, sect. Peninsularis, Mexico, Baja California

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84. SECT. PENINSULARIS

Perennial or suffruticose herbs or shrubs 1.0-3.0 m high; stems pubescent with short or elongate mostly simple hairs; leaves mostly 4-9 cm long, 2-4 cm wide; petioles 1-2 cm long; blades lanceolate to sub-deltoid, sparsely to densely pubescent above and below, mainly along the venation, surfaces glandular-punctate, the margins crenulate to serrate; spikes 4-20 cm long, congested to interrupted; flowers 12-30 mm long, 4-16 to a node, the pedicels 2-5 mm long; floral bracts linear-lanceolate, soon deciduous, rarely not; calyces (flowering) 4-9 mm long, lips short, the upper lip 3-7-veined; corollas purple, the tubes 8-20 mm long; upper lips 3-13 mm long; stamens included; stylar shafts flattened, pilose, the upper branches 2-4 times as long as the lower; nutlets ovate, glabrous, ca 2 mm long, 1 mm wide.
TYPE: Salvia peninsularis Brandegee.

Key to species

1. Leaves markedly bicolored; stems pubescent with dense cottony-
   white hairs...**S. moranii**
1. Leaves not bicolored; stems not as described in the above...........**(2)**

2. Flowers 12-15 mm long; La Paz and southwards...........**S. lapazana**
2. Flowers 20-25 mm long; Sierra de la Giganta and northwards.........
   ....**S. peninsularis**

SALVIA LAPAZANA B.L. Turner, **sp. nov. Fig. 1, 3**

       Salviae peninsulari Brandgee similis sed differt habitu fruticoso 1.5-3.0 m in altitudinis (vs herbis perennis usque ad 1 m), foliis minoribus, calycibus (asdfasd) minoribus (5-6 mm longis vs 8-10 mm), et corollis minoribus (11-14 mm longis vs ca 30 mm), et bracteis floralibus majoribus lineari-lanceolatis persistentibus.

Perennial suffruticose herbs or shrubs to 3 m high. **Stems** minutely puberulent with appressed hairs. **Leaves** mostly 3-5 cm long; petioles 1-2 cm long, pubescent below with appressed hairs, mostly along the venation, surfaces glandular-punctate, the margins serrulate. **Flowers** 12-15 mm long, 6-12 to a node. **Floral bracts** linear-lanceolate, soon deciduous. **Spikes** congested, 4-6 cm long, sessile or nearly so. **Calyces** (flowering) 4-6 mm long, minutely appressed-pubescent, the upper lip 5-veined. **Corollas** purple, 10-15 mm long; tubes 8-10 mm long; upper lips 3-4 mm long, somewhat shorter than the lower. **Nutlets** ovoid, glabrous, ca 1.5 mm long, 1.0 mm wide.

TYPE: **MEXICO. BAJA CALIFORNIA SUR: Mpio. La Paz**, "Los Limpios, Sierra La Laguna, al E de Todos Santos, Selva baja caducifolia alterada, Suelo arenoso amarillo." 18 Oct 1985, *P. Tenorio L. 10585* (Holotype: TEX) [con C. Romero de T., J.I. Solis y J. Agudes E.]

Additional Specimen Examined (sterile): Same Mpio. as above: "Arroyo San Bartolo, 2.5 km above San Bartolo, 400 m, ca 23 43.5 N, 109 53 W, common in wash, "shrub to 3 m tall." 8 Jan 1959, *Moran 6953* (CAS).

The present novelty is clearly related to *S. peninsularis* but is smaller in all parts; it also occupies a distinct geographical area and vegetational type.

The taxon is named for the Mpio. La Paz, where first obtained.

SALVIA MORANII B.L. Turner, **sp. nov., Fig. 2, 3**

Salviae peninsulari Brandegee similis sed differt habitu subfruticoso usque ad 1.5 m in altitudinis (vs. herbis perennis usque ad 1 m), caulibus vestimento trichomatorum albo-gossypinorum, foliis bicoloribus, et calycibus valde 7-nervatis (vs. 3-nervatis).

"Lax shrub 1 ½ m. tall." **Stems** densely pubescent with white-cottony hairs. **Leaves** mostly 6-9 cm long, 2-3 cm wide; petioles 0.5-1.2 cm long; blades ovate to ovate-lanceolate, penninervate, bicolored, the lower surfaces densely white-pubescent, the margins minutely serrulate. **Capitulescence** terminal, 6-10 cm long, 4-10 flowers to a node, the pedicels 1-4 mm long. **Floral bracts** ovate, soon deciduous. **Calyces** (flowering) 10-12 mm long, 5-7 mm across; pubescent like the stems; lips obtuse, ca equal in length, the upper lip markedly 7- veined. **Corollas** ca 3 cm long, "rose-purple," the upper lips ca 12 mm long, pilose with purple hairs, ca as long as the reflexed lower lip. **Stamens** with anthers included. **Stylar shafts** pilose, the upper branch 2-3 times as long as the lower. **Nutlets** not observed.

TYPE: MEXICO. BAJA CALIFORNIA SUR: La Aguja, 1900 m, ca 23 34 N, 110 01 W, 18 May 1959, *Reid V. Moran 7449* (Holotype: CAS).

Moran identified the above type as "Salvia marci Epling vel aff." I treat the latter as a synonym of *S. peninsularis*, as noted below. *Salvia moranii* is markedly distinct from *S. peninsularis*, as noted in the Latin diagnosis; indeed, it might well belong to yet another section of Salvia. I have included it herein largely on the basis of its overall gestalt, and geography.

The species is named for the well-known American botanist, Reid Moran, perhaps best known for his fieldwork in the Southwestern

U.S.A., his wit (cf. Thomas 1977). and his penchant for making light of the pretentious systematic practitioner (System. Bot. 28: 191-207. 2003.).

SALVIA PENINSULARIS Brandegee, Zoe 5: 108. 1901.
Salvia marci Epling ex M.E. Jones

Perennial or suffruticose herbs 1.0-1.5 m high; leaves mostly 6-11 cm long, 2-4 cm wide; petioles 1-2 cm long; blades lanceolate to sub-deltoid, spikes 6-20 cm long, congested to interrupted (with age); flowers 20-30 mm long, 4-14 to a node, the pedicels 2-4 mm long; calyces (flowering) 8-9 mm long, lips short, the upper lip 5-7-veined, pubescent like the stems; corollas purple, the tubes 10-20 mm long; upper lips 6-9 mm long, as long as, or somewhat longer, than the lower; nutlets, glabrous, somewhat mottled, ca 2 mm long, 1 mm wide.

Epling (1939) suggested that *Salvia marci* might be the same as the present taxon; he subsequently reduced it to synonymy (Epling and Jativa 1968), as noted in the above listing. Wiggins (1980), however, retained the latter, restricting this to central Baja California, *S. peninsularis* confined to the Sierra Gigantea.

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Wiggins, I.L. 1980. *Salvia*, in Fl. Baja Calif. pp. 430-434.

Fig. 1. Holotype of *Salvia lapazana*.

Fig. 2. Holotype of *Salvia moranii*.
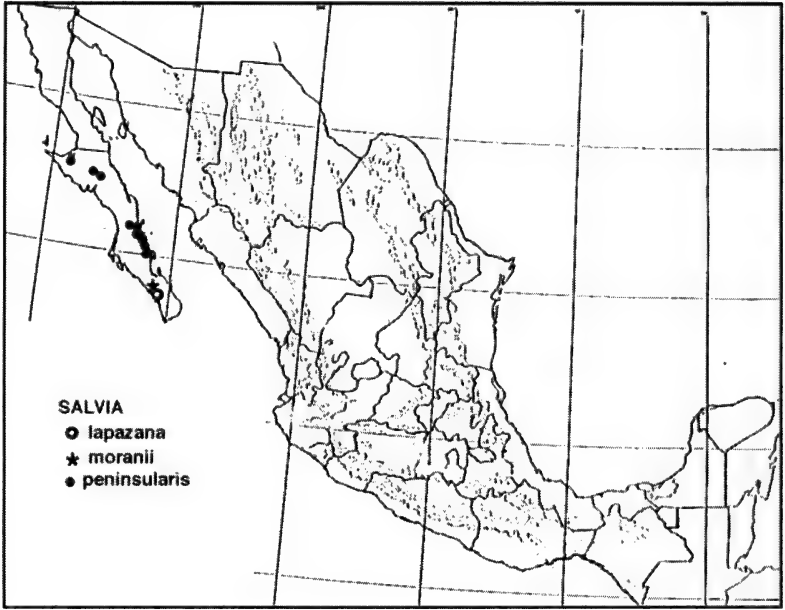
Fig. 3. Distribution of *Salvia lapazana*, *S. moranii* and *S. peninsularis*.

INFRASPECIFIC CATEGORIES IN *THELESPERMA FILIFOLIUM* (ASTERACEAE: COREOPSIDEAE)

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ABSTRACT

Based largely upon the doctoral dissertation of Melchert (1963), *Thelesperma filifolium* is accepted as having two widely distributed infraspecific categories: var. *filifolium* in the eastern part of its range; var. *intermedium* in the western part. Populations of the two taxa intergrade near regions of contact, making varietal identifications in such areas difficult. Distinctions between the two taxa are called to the fore, and an account of their chromosomal variation is given. Maps showing the distribution of the complex are provided. *Phytologia 92(1): 27-30 (April, 2010).*

KEY WORDS: Asteraceae, Coreopsideae, *Thelesperma filifolium*, *T. flavodiscum*, Texas, chromosomes.

Strother (2006) treated *Thelesperma* for the Flora of North America. In this he called attention to the fact that some workers recognized a var. *intermedium* (Rydb.) Shinners within the *T. filifolium* (Hook.) A. Gray complex, Rydberg having described this initially as a species; Shinners subsequently reducing this to varietal rank. Melchert (1963), in his unpublished doctoral thesis, accepted the treatment of Shinners, noting that the two taxa are quite distinct: populations with yellow disc florets and bright yellow rays having relatively broader leaf segments (var. *intermedium*) occurring in the more western portions of the distribution of the species; the typical var. *filifolium* with non-yellow disc florets occurring in the more eastern portion of its range (Fig. 1). Melchert correctly noted that in those areas where the two taxa overlap, populational intergradation occurs. Strother (2006), unfortunately, did not cite Melchert's work, although he called attention

to the varietal differences noted by Melchert, as well as the distribution of each.

Melchert (1963) understood the *T. filifolium* complex quite well. Indeed, he collected numerous populations of the species from throughout most of its Texas distribution (fig. 2), including chromosome counts from over 50 populations. Chromosome numbers of var. *filifolium* (13 populations), were found to be 2n = 16, only one population counted as 2n = 18. Chromosome counts for the var. *intermedium* were more varied: 19 with 2n = 16; 17 with 2n =18; none with 2n = 20; two with 2n =22; and one population having individuals with counts of both 2n = 16 and18. Strother (2006) noted the chromosome counts of *T. filifolium* as "2n = 16, 18," these presumably from counts taken from the literature (i.e., without consultation of Melchert's thesis).

Thelesperma filifolium clearly contains two well-marked, regional varieties, much as remarked upon by Melchert. Populations of the two varieties intergrade in regions of near contact, this readily seen in traversing the region of overlap along IH 10 between Sonora and Ozona, Texas. In the Sonora area, most of the populations contain *filifolium* type plants, with a few individuals being *intermedium* types; in the Ozona area the populations become otherwise, being predominantly composed of var. *intermedium* type plants. Westward and eastwards from the region of contact, the populations shift by degrees towards the more uniform populations to be expected. Occasionally, a plant resembling one variety or the other may crop up within regions of otherwise morphological homogeneity. But such are the vagaries of evolution and gene flow!

Clearly, the two taxa are worthy of more extended study. It is possible that some genetic contamination between *T. filifolium* and *T. flavodiscum* (Shinners) B.L. Turner has occurred in the recent past in the more eastern portions of the distribution of var. *filifolium*, at least to judge from the habit and vegetative features of occasional plants referred to var. *filifolium* by both Melchert and myself. But, as already noted (Turner 2007), the distinctions between *T. flavodiscum* and *T. filifolium* are not subtle, as maintained by Strother (2006), and are not "better treated as one species," as he suggested might be the case.

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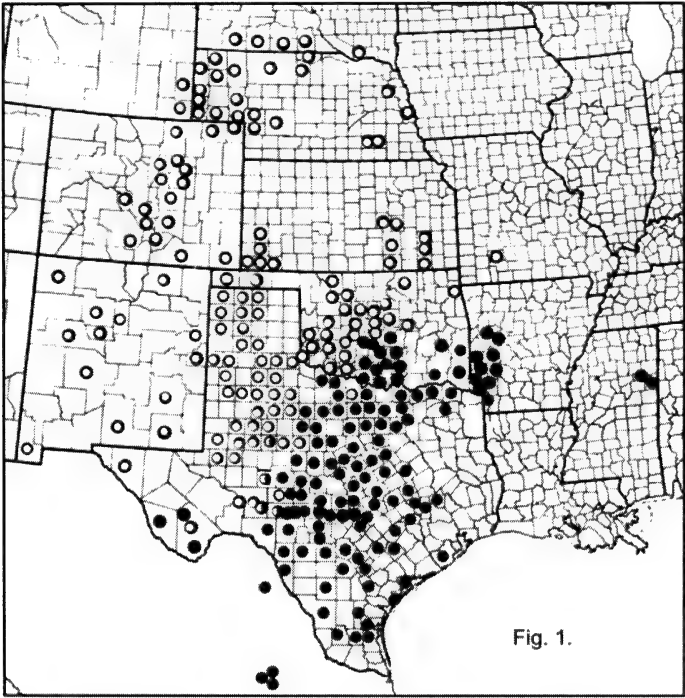
Fig. 1. Distribution of *Thelesperma filifolium*: var. *filifolium* (closed circles); var. *intermedium* (open circles); intermediate populations (half circles). Mexican populations are believed to have been recently introduced (Melchert, in prep.).
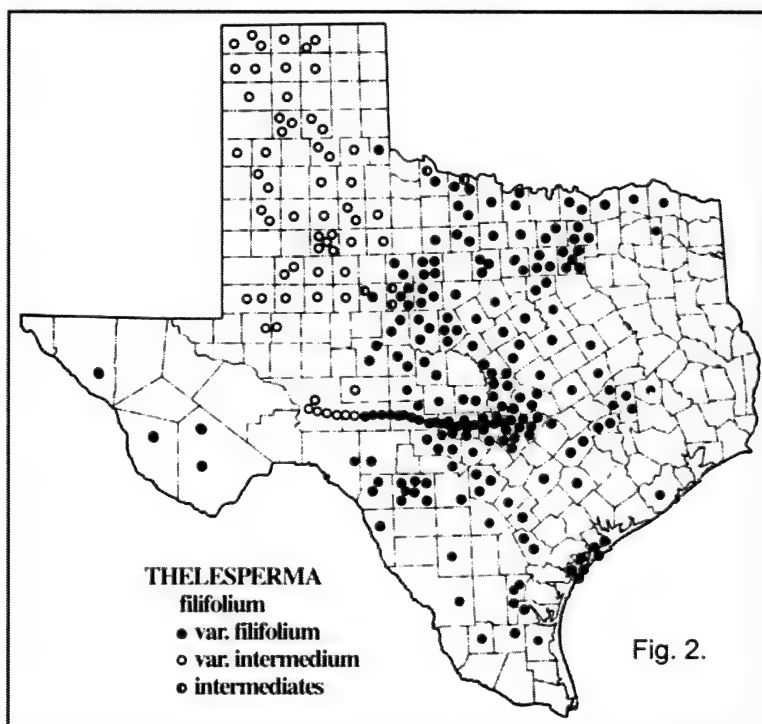
Fig. 2. Distribution of *Thelesperma filifolium*: var. f*ilifolium* in Texas
(closed circles); var. *intermedium* (open circles); intermediate
populations (half circles).

GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS CEDRUS* WEBB. & BERTHOL. FROM MADEIRA AND THE CANARY ISLANDS.

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ABSTRACT

The volatile leaf oils of *J. cedrus* from Madeira, Gran Canaria, La Gomera, La Palma and Tenerife were analyzed and the compositions reported and compared with *J. oxycedrus*, France. The oil of *J. cedrus* from Madeira Island was moderately high in α-pinene (24.9%), limonene (10.9%), β-phellandrene (7.3%), δ-3-carene (5.6%), (E)-caryophyllene (4.3%), with considerable amounts of diterpenes: sandaracopimara-8(14),15-diene (2.8%), abietatriene (1.4%), iso-abienol (7.2%), nezukol (0.4%), sempervirol (0.6%), trans-totarol (8.6%), and trans-ferruginol (1.2%). In contrast, *J. cedrus* from the Canary Islands was very high in α-pinene (54.6 - 66.3%), with moderate amounts of β-pinene (2.2-3.3%), myrcene (3.6-5.9%), α-phellandrene (0.5-3.0%), β-phellandrene (4.1-13.1%), with little or no δ-3-carene (0.0-0.1%), and almost no diterpenes. The major geographic trend was the divergence of *J. cedrus*, Madeira, from the

populations in the Canary Islands. The level of divergence of the Madeira population from the Canary Islands populations was comparable to the divergence of *J. cedrus* from *J. oxycedrus*, implying considerable evolutionary differences. *Phytologia 92(1): 31-43 (April, 2010).*

KEY WORDS: *Juniperus cedrus, J. oxycedrus,* Cupressaceae, Madeira Island, Canary Islands, essential oil composition, α-pinene, δ-3-carene, β-phellandrene, isoabienol.

Juniperus cedrus Webb & Berthol. is endemic to Madeira and the Canary Islands (Adams, 2008) where it grows naturally on several islands (Fig. 1). Wild populations of *J. cedrus* are very rare on Madeira Island. Nobrega (pers. comm.) reported that he found 1 tree near the summit of Pico Ferreiro (1583 m) and 16 trees near the summit of Pico das Torres (1847 m), although it may grow on some nearby peaks. Fortunately, seeds from natural *J. cedrus* populations have been germinated and *J. cedrus* plants have been widely cultivated on Madeira. *Juniperus cedrus* is also generally rare in the Canary Islands.

Natural populations are found on Gran Canaria (only a few plants are known from Montaña del Cedro), La Palma (several hundred plants, near the summit of La Caldera de Taburiente), Tenerife (several hundred plants, El Teide National Park), and La Gomera (only a few plants are known from Garajonay National Park).

Adams (2000) reported (TIC values) that the leaf oils of *J. cedrus* from Tenerife contained large amounts of α-pinene (70.7%), with moderate amounts of myrcene (6.3%), limonene (4.5%), β-phellandrene (4.6%), β-pinene (4.1%), no δ-3-carene and no diterpenes except abietatriene (0.1%). Cavaleiro et al. (2002) analyzed 10 cultivated *J. cedrus* trees from Madeira and reported (FID values): 19.6 - 55.3% α-pinene, 17.3-32.7% limonene, 5.5-15.7% δ-3-carene, with moderate amounts of diterpenes (abietatriene, 0.1-0.8%; sandaracopimaradiene, 0.1-6.1%; isoabienol, 0.5-1.3%; and trans-totarol (0.4-2.2%). They commented that these differences (irrespective of TIC vs. FID quantitation), seem to indicate some geographical chemical variability between Madeira and the Canary

Islands. Pino et al. (2003) analyzed a single cultivated tree at the Agriculture Dept., Camacha, Madeira and they reported (FID values) the oil contained 36.0% α-pinene, 21.1% limonene, 14.8% δ-3-carene, 3.8% β-caryphyllene (= (E)-caryophyllene), as well as several diterpenes (abietatriene, 1.2%; iso-abienol, 0.4%, mis-identified as sclareol; sempervirol, 0.7%, mis-identified as cis-totarol; and trans-totarol, 0.6%). In general, their results agreed with Cavaleiro et al. (2002).
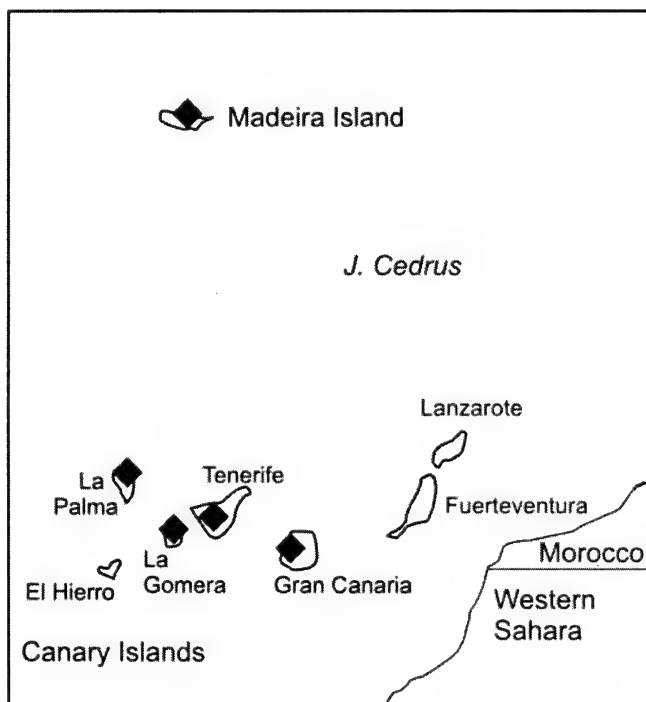
Figure 1. Distribution of *J. cedrus* from all known populations.

Thus, it appears that *J. cedrus* from Madeira may be differentiated from *J. cedrus* of the Canary Islands. The purpose of this paper is to investigate geographic variation in the volatile leaf oils of *J. cedrus* plants from throughout its range, analyzed under identical conditions.

The Madeira juniper was described as *J. oxycedrus* L. subsp. *maderensis* Menezes (Bull. Acad. Int. Georg. Bot. 18: xii. 1908), then Rivas Mart. transferred it to *Juniperus cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al. [Itinera Geobot. 15(2): 703 (2002)].

Farjon (2005) noted that leaf size and shape vary considerable in *J. cedrus*. In view of the differences in oils reported by Adams (2000, from Tenerife), and Cavaleiro et al. (2002, from Madeira), a study was undertaken to collect *J. cedrus* from all known populations on both Madeira and the Canary Islands. *Juniperus oxycedrus* from France was included in the study. The purpose of this paper is to present these results.

MATERIALS AND METHODS

Plant material - *J. cedrus*. Madeira Island: cultivated at Agriculture Dept., Camacha, 32° 40.374'N, 16° 50.834'W, 650 m, (= tree analyzed by Pino et al. (2003), *Adams 11496*; 32° 41.871'N, 16° 52.986'W, 1143 m, (= trees 1-5 analyzed by Cavaleiro et al. (2002), *Adams 11497-11501,* Canary Islands: Gran Canaria Island, Montaña del Cedro, 27° 57'N, 15° 44'W, 850 m, collected by Beatrice Rumeu, *Adams 11505-11507*; La Palma Island, Piedra Llana and Caldera de Taburiente, El Tiedre, 28° 45.069'N, 17° 50.150'W, 2160 m, *Adams 11509-11513*; Tenerife Island, Riscos de La Fortaleza, 28° 18.868'N, 16° 35.975'W, 2150 m, *Adams 11518-11522*; La Gomera Island, Garajonay National Park, 28° 6.544'N, 17° 13.533'W, 1339 m, *Adams 11523-11527*. *J. oxycedrus*. 4 km e of Forcalquier, France, 44° 04.06'N, 5° 59.19' E, 490 m, *Adams 9039-9041*.   Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh leaves (200 g.) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (48h, 100°C) for determination of oil yields.

Analyses - Oils from 3-6 trees from each population were analyzed and average populational values are reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to

a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 1991 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Table 1 shows that the oil composition of *J. cedrus* [from re-sampling the trees 1-5 of Cavaleiro et al. (2002) plus the single tree of Pino et al. (2003)] from Madeira Island was moderately high in α-pinene (24.9%), limonene (10.9%), β-phellandrene (7.3%), δ-3-carene (5.6%), (E)-caryophyllene (4.3%), with considerable amounts of diterpenes: sandaracopimara-8(14),15-diene (2.8%), abietatriene (1.4%), iso-abienol (7.2%), nezukol (0.4%), sempervirol (0.6%), trans-totarol (8.6%), and trans-ferruginol (1.2%). In contrast, *J. cedrus* from the Canary Islands (table 1) was very high in α-pinene (54.6 - 66.3%), with moderate amounts of β-pinene (2.2-3.3%), myrcene (3.6-5.9%), α-phellandrene (0.5-3.0%), β-phellandrene (4.1-13.1%), with little or no δ-3-carene (0.0-0.1%), and almost no diterpenes. The report of Cavaleiro et al. (2002) is borne out. There are considerable differences between the oils of *J. cedrus* from Madeira and the Canary Islands.

The oil of *J. oxycedrus*, France, was very high in α-pinene (62.1%), with moderate amounts of δ-3-carene (4.3%), limonene (2.8%), manool oxide (4.1%) with considerable no diterpenes.

There were also some differences in *J. cedrus* among the Canary Islands (table 1). For example, the Tenerife plants have large amounts of β-phellandrene (13.1%). However, the overall similarities among these oils are difficult to visualize. Principal coordinate analysis (PCO) based on 43 terpenoids resulted in eigenroots accounting for 60.55, 18.27, 11.90 and 9.28% of the variance among the island samples. Ordination of the samples reveals that the major trend (42% of the variance) is due to the differentiation of the Madeira plants from *J. cedrus* of the Canary Islands and *J. oxycedrus* (Fig. 2). The second axis (29%) separated *J. oxycedrus*, France, from Madeira and Canary Island plants. The third axis (14%) separated *J. cedrus* from the Canary Islands from Madeira and *J. oxycedrus* (Fig. 2).
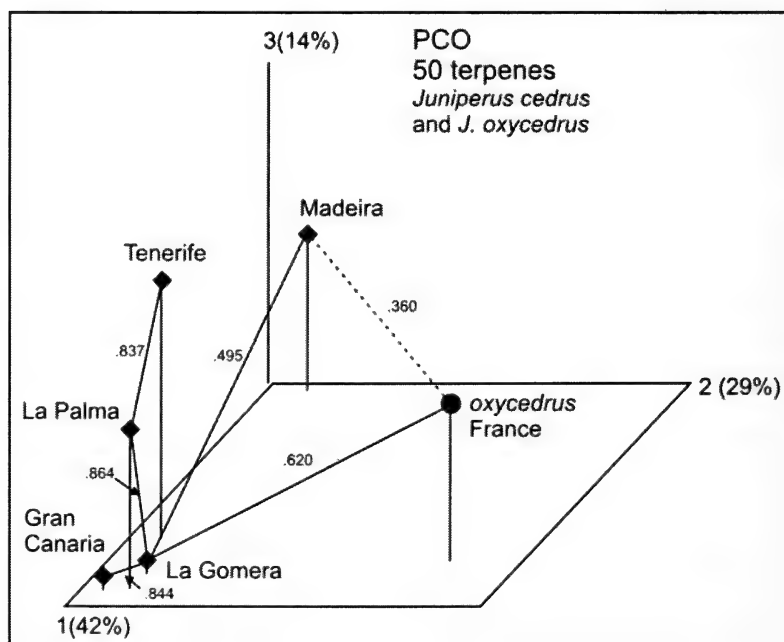
Figure 2. PCO based on 50 terpenes. Note the differentiation of *J. cedrus*, Madeira from the Canary Island populations.

To better understand the scale of geographic variation, a minimum spanning network was constructed and overlaid on a map of

the islands (Fig. 3). Because the similarities were computed by the absolute difference divided by the maximum for a compound, the differences are magnified. For example, the similarity of Madeira to La Gomera (0.496) does not mean that these samples share only 49.6% of the components (see Table 1). Nevertheless, one can see that the largest geographical distance (Madeira to the Canary Islands) corresponds to the greatest amount of differentiation. And, conversely, oils of *J. cedrus* from the four Canary Islands are all very similar (0.837 - 0.864, Fig. 3).

Madeira
Island

Minimum Spanning
Network
50 terpenoids

.495

Lanzarote

La Palma .833 Tenerife
.864
Fuerteventura

El Hierro La Gomera .844 Gran Canaria

Morocco

Western Sahara
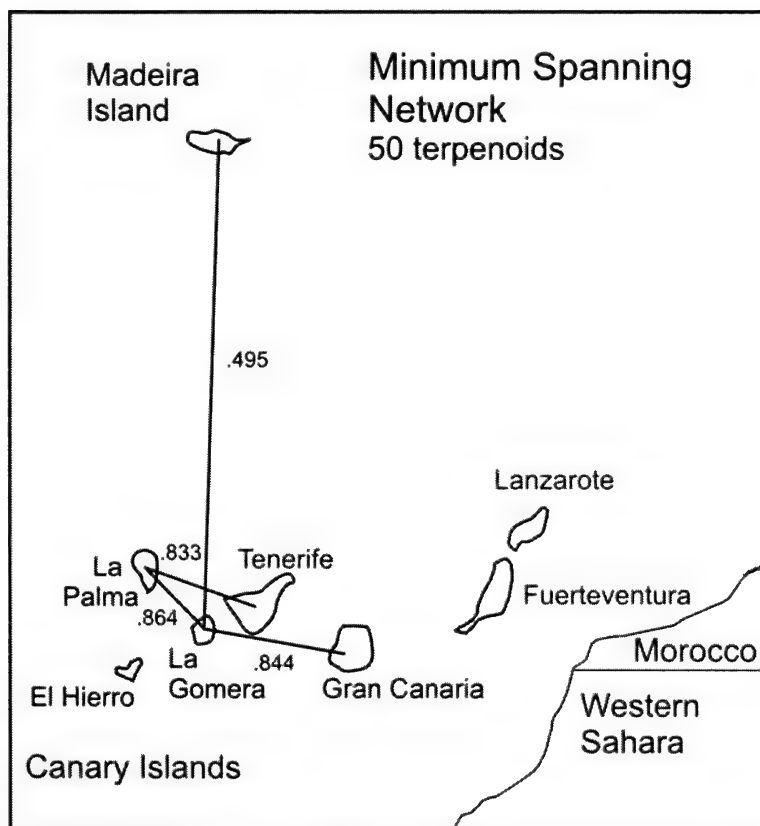
Canary Islands

Figure 3. Minimum spanning network showing the divergence of the *J. cedrus* from Madeira from Canary Island populations.

Phylogenetically, Adams (2008) has shown that, based on nrDNA and cp trnC-trnD sequence data, *J. cedrus* is most closely related to *J. macrocarpa* (Spain) and *J. oxycedrus* (Spain) and not as related to *J. brevifolia* (Azores). It appears that *J. cedrus* may have evolved from a *J. macrocarpa/ oxycedrus* ancestor from Spain or even Morocco, as *J. oxycedrus* presently grows nearby in Morocco.

Juniperus cedrus from Madeira has been treated as a subspecies (*J. cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al.). The volatile leaf oil composition gives support for the recognition of an infraspecific taxon of *J. cedrus* from Madeira.

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Table I. Composition of the leaf oils of *J. cedrus* from Madeira (Mad) and the Canary Islands: Gran Canaria (GC), Tenerife (Ten), La Palma (LP), La Gomera (LG) and *J. oxycedrus*, France (Oxy).

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
859	(E)-2-hexenal	0.4	0.2	0.5	0.7	1.5	0.2
921	tricyclene	0.1	0.1	0.2	0.1	0.1	0.1
924	α-thujene	0.1	t	t	t	t	t
932	**α-pinene***	**24.9**	**60.9**	**54.6**	**66.3**	**65.3**	**62.1**
945	α-fenchene	0.3	0.2	t	t	t	0.2
946	camphene*	0.1	0.3	0.4	0.4	0.4	0.4
953	thuja-2,4(10)-diene	-	-	-	-	-	0.1
969	sabinene	0.1	t	0.3	t	0.2	0.4
974	1-octen-3-ol	0.3	0.1	t	0.3	0.3	t
974	**β-pinene***	**0.7**	**3.3**	**3.1**	**3.4**	**2.2**	**1.7**
988	**myrcene***	**1.2**	**5.3**	**5.9**	**4.5**	**3.6**	**2.2**
1001	δ-2-carene	0.2	0.1	0.2	t	0.1	0.1
1002	**α-phellandrene***	**-**	**1.1**	**3.0**	**0.5**	**0.5**	**0.1**
1008	**δ-3-carene***	**5.6**	**-**	**-**	**0.1**	**0.1**	**4.3**
1020	p-cymene*	0.1	0.5	0.8	0.2	0.3	0.3
1024	**limonene***	**10.9**	**3.0**	**1.5**	**2.0**	**3.0**	**2.8**
1025	**β-phellandrene***	**7.3**	**6.1**	**13.1**	**4.1**	**4.6**	**0.8**
1044	(E)-β-ocimene	0.1	-	-	-	0.1	t
1054	γ-terpinene	0.1	-	t	t	-	0.1
1063	n-octanol*	-	0.1	0.1	0.3	-	-
1086	terpinolene*	0.7	0.4	1.2	0.5	0.4	0.6
1095	linalool	-	-	-	-	-	0.1
1100	n-nonanal*	t	0.3	0.2	0.2	0.2	t
1110	**1-octen-3-yl acetate***	**0.5**	**-**	**0.1**	**0.2**	**t**	**t**
1118	cis-p-menth-2-en-1-ol	-	-	0.2	0.1	-	-
1120	3-octanyl acetate	t	-	-	-	t	t
1122	α-campholenal*	-	0.1	t	0.1	-	0.6
1135	trans-pinocarveol	-	0.1	0.1	0.1	-	0.4
1141	camphor	t	0.2	0.1	0.2	t	-
1165	borneol	-	0.2	t	0.2	t	-
1174	terpinen-4-ol	0.1	0.1	t	0.2	t	0.1
1178	naphthalene	0.1	0.1	0.1	0.1	0.2	0.1
1179	p-cymen-8-ol	t	t	t	0.1	-	t

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
1186	α-terpineol*	0.1	0.3	0.3	0.3	0.2	0.4
1195	myrtenal	-	-	t	t	-	0.1
1232	**thymol, methyl ether***	**-**	**0.3**	**t**	**t**	**0.1**	**-**
1241	**carvacrol, methyl ether***	**0.6**	**-**	**-**	**-**	**-**	**-**
1287	bornyl acetate*	0.4	0.1	0.7	0.9	0.1	0.4
1292	(E,Z)-2,4-decadienal	t	-	t	0.1	t	t
1298	**carvacrol***	**0.3**	**-**	**-**	**-**	**-**	**-**
1309	**p-vinyl guiacol***	**0.1**	**-**	**-**	**-**	**-**	**-**
1315	(E,E)-2,4-decadienal	0.1	0.1	0.1	0.1	t	-
1324	myrtenyl acetate	-	-	0.1	t	-	-
1346	**α-terpinyl acetate***	**1.4**	**0.3**	**3.5**	**0.3**	**0.3**	**t**
1373	**α-ylangene***	**0.1**	**-**	**-**	**-**	**-**	**-**
1379	geranyl acetate	-	-	0.1	t	t	-
1387	β-cubebene	0.1	t	0.1	t	t	-
1387	β-bourbenone	-	-	-	-	-	0.2
1400	**β-longipinene***	**0.5**	**-**	**-**	**-**	**-**	**-**
1411	**cis-α-bergamotene***	**0.1**	**-**	**-**	**-**	**-**	**-**
1417	**(E)-caryophyllene***	**4.3**	**0.5**	**2.7**	**2.0**	**1.0**	**0.3**
1429	**cis-thujopsene***	**1.0**	**-**	**-**	**-**	**-**	**-**
1448	cis-muurola-3,5-diene	t	-	t	t	t	-
1452	α-humulene*	1.0	0.2	0.7	0.6	0.3	0.2
1469	n-dodecanol	-	-	0.1	0.1	-	-
1475	γ-gurjunene	0.2	-	-	-	-	-
1478	γ-muurolene	-	-	-	-	-	0.1
1480	**germacrene D***	**0.7**	**0.1**	**0.8**	**0.3**	**t**	**1.5**
1493	trans-muurola-4(14),5-diene	-	-	t	0.1	-	-
1493	**epi-cubebol***	**0.7**	**0.1**	**t**	**0.1**	**0.3**	**-**
1495	2-tridecanone	-	-	-	-	-	0.1
1500	α-muurolene	0.1	-	-	-	-	-
1505	E,E-α-farnesene	-	-	t	0.1	-	-
1513	**γ-cadinene***	**0.4**	**-**	**-**	**-**	**-**	**0.5**
1513	cubebol*	0.4	0.3	0.1	0.3	0.8	-
1522	δ-cadinene*	0.6	0.2	0.2	0.4	0.5	0.3
1533	trans-cadina-1,4-diene	-	-	t	t	-	-
1548	elemol	-	t	t	0.1	-	-

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
1559	germacrene B	-	0.1	0.1	0.1	0.2	t
1561	(E)-nerolidol*	0.1	0.9	0.2	0.2	0.2	-
1582	caryophyllene oxide*	2.3	3.1	1.3	2.0	1.6	0.3
1594	ethyl dodecanoate	-	t	0.2	0.1	-	-
1594	**salvial-4(14)-en-1-one**	**-**	**-**	**-**	**-**	**-**	**0.2**
1600	hexadecane	-	-	-	0.1	-	0.2
1600	cedrol	0.1	t	t	0.1	t	-
1608	humulene epoxide II*	0.6	0.8	0.3	0.2	0.2	0.6
1627	1-epi-cubenol*	0.5	0.6	0.1	0.3	0.6	-
1638	epi-α-cadinol*	t	-	-	-	-	0.7
1638	epi-α-muurolol	t	-	-	-	-	0.2
1639	caryophyllla-4(12),8(13)-dien-5-α-ol	-	0.1	t	0.1	-	-
1649	β-eudesmol	-	t	t	0.1	-	0.2
1652	α-eudesmol	-	t	-	t	-	-
1652	**α-cadinol***	**t**	**t**	**0.2**	**t**	**-**	**0.8**
1667	**(6Z)-pentadecen-2-one***	**-**	**-**	**-**	**-**	**-**	**0.9**
1685	germacra-4(15),5,10(14)-trien-1-al	0.3	0.1	0.1	0.1	t	0.9
1700	heptadecane	0.1	t	t	0.1	-	0.2
1713	(2E,6Z)-farnesal	-	-	-	-	-	0.3
1740	(2E,6E)-farnesal	-	-	-	-	-	0.3
1800	octadecane	t	-	t	t	-	0.1
1900	nonadecane	t	-	t	t	-	t
1929	**C$_{20}$, 41, 55, 257, 270 (isopimaridiene?)***	**0.8**	**-**	**-**	**-**	**-**	**-**
1968	**sandaracopimara-8(14),15-diene***	**2.8**	**-**	**-**	**-**	**-**	**0.1**
1987	**manool oxide***	**-**	**-**	**-**	**-**	**-**	**4.1**
2009	**epi-13-manool oxide**	**-**	**-**	**-**	**-**	**-**	**0.2**
2022	abieta-8,12-diene	-	-	-	-	-	0.1
2055	abietatriene	1.4	0.8	t	0.6	2.7	1.0
2087	**abietadiene***	**0.1**	**-**	**-**	**-**	**-**	**1.0**
2105	**isoabienol***	**7.2**	**-**	**-**	**-**	**-**	**-**
2132	**nezukol***	**0.4**	**-**	**-**	**-**	**-**	**-**
2184	**sandaracopimarinal***	**-**	**-**	**-**	**-**	**-**	**0.3**
2189	**1-docosene**	**-**	**-**	**-**	**-**	**-**	**0.2**
2200	**docosane**	**-**	**-**	**-**	**-**	**-**	**0.2**

AI	Compound	Mad	GC	Ten	L P	LG	Oxy
2200	**C_{20}-OH, <u>41</u>,135, 270,288***	**0.9**	-	-	-	-	-
2218	**(E)-phytol acetate**	-	-	-	-	-	**0.2**
2282	**sempervirol***	**0.6**	-	-	-	-	-
2300	tricosane	-	-	0.1	0.1	-	0.3
2314	**trans-totarol***	**8.6**	**0.5**	-	**0.1**	**2.3**	-
2331	trans-ferruginol	1.2	0.4	-	-	0.9	-

AI = Arithmetic Index on DB-5 column. *Used in numerical analyses.
Compositional values less than 0.1% are denoted as traces (t).
Unidentified components less than 0.5% are not reported. Those
compounds that appear to distinguish taxa are in boldface.

SPECIATION OF *JUNIPERUS CEDRUS* AND *J. MADERENSIS* IN THE ARCHIPELAGOS OF CANARIES AND MADEIRA BASED ON TERPENOIDS AND nrDNA AND petN-psbM SEQUENCES.

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ABSTRACT

Analyses of nrDNA and petN-PsbM sequence data, combined with leaf terpenes data, revealed that putative *J. cedrus* from Madeira is as distinct from *J. cedrus* of the Canary Islands as it is from *J. oxycedrus*. These data support the recognition of the Madeira juniper at the specific level, *J. maderensis* (Menezes) R. P. Adams **comb. et stat. nov.** *Phytologia 92(1): 44-55 (April, 2010).*

KEY WORDS: *Juniperus cedrus, J. maderensis, J. oxycedrus, J. macrocarpa, J. brevifolia,* Cupressaceae, Madeira Island, Canary Islands, nrDNA, cp petN-psbM, taxonomy.

In a previous paper of this issue, we reported on the leaf oils of *Juniperus cedrus* Webb & Berthol. from Madeira and the Canary Islands (Adams et al. 2010). The leaf oils were found to differ greatly from putative *J. cedrus* in Madeira to those of *J. cedrus* of the Canary Islands (Fig. 1). In fact, the oils from *J. cedrus* of the Canary Islands were more similar to the *J. oxycedrus* oil (0.620, Fig. 1) than to putative *J. cedrus* oil from Madeira (0.495, Fig. 1).
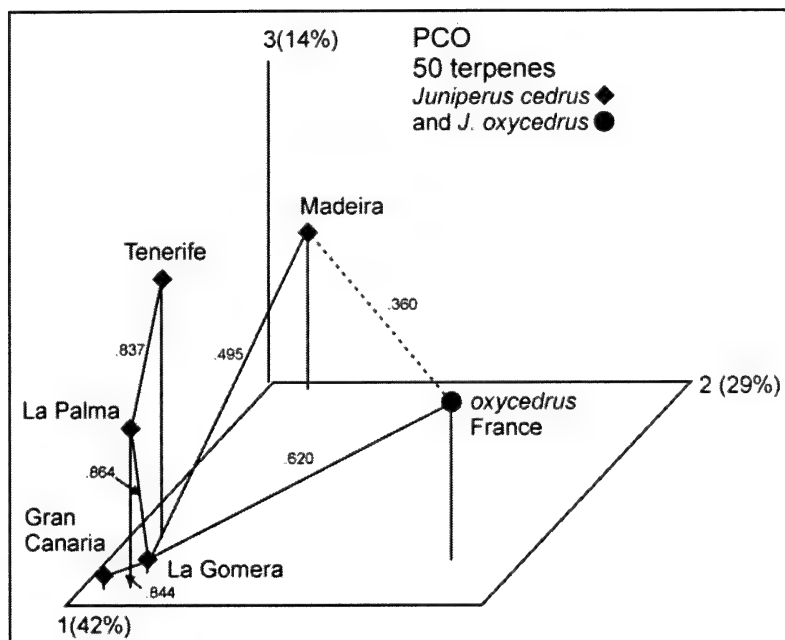
Figure 1. PCO based on 50 terpenes. Note the differentiation of *J. cedrus*, Madeira from the Canary Island populations. Numbers next to lines are similarities. Adapted from Adams et al. (2010).

The Madeira juniper was described as *J. oxycedrus* L. subsp. *maderensis* Menezes (Bull. Acad. Int. Georg. Bot. 18: xii. 1908). Recently the taxon was transferred to *Juniperus cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al. [Itinera Geobot. 15(2): 703. 2002]. Farjon (2005) noted that leaf size and shape vary considerably in *J. cedrus* and concluded that plants from Madeira were allied with *J. cedrus* and not *J. brevifolia*.

Adams (2008) reported that *J. cedrus* of the Canary Islands, is in a clade with *J. oxycedrus* and *J. macrocarpa*, not in the distinct clade of *J. brevifolia*, *J. navicularis* and *J. deltoides* (Fig. 2).
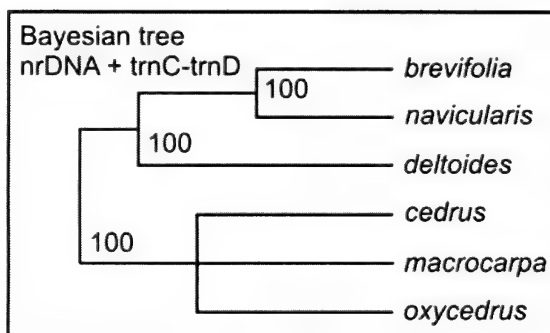
Figure 2. Partial Bayesian tree based on nrDNA + trnC-trnD sequences. Numbers at the nodes are posterior probabilities on a percent basis.

The purpose of this paper is to examine DNA sequence data of the nrDNA and petN-spacer-psbM cp regions to resolve the taxonomic affinities of *J. cedrus* from the Canary Islands and putative *J. cedrus* from Madeira.

MATERIALS AND METHODS

Plant material (GenBank accessions: nrDNA, petN-psbM) - *J. cedrus* (*11497*, GU139568, GU139573; *11519*, GU139569, GU139574). Madeira Island: cultivated at Agriculture Dept., Camacha, 32° 40.374'N, 16° 50.834'W, 650 m, (= tree analyzed by Pino et al. 2003), *Adams 11496*; 32° 41.871'N, 16° 52.986'W, 1143 m, (= trees 1-5 analyzed by Cavaleiro et al. 2002), *Adams 11497-11501*; Canary Islands: Gran Canaria Island, Montaña del Cedro, 27° 57'N, 15° 44'W, 850 m, collected by Beatriz Rumeu, *Adams 11505-11507*; La Palma Island, Piedra Llana and La Caldera de Taburiente, 28° 45.069'N, 17° 50.150'W, 2160 m, *Adams 11509-11513*; Tenerife Island, Riscos de La Fortaleza, 28° 18.868'N, 16° 35.975'W, 2150 m, *Adams 11518-11522*; La Gomera Island, Garajonay National Park, 28° 6.544'N, 17° 13.533'W, 1339 m, *Adams 11523-11527*. *J. brevifolia*, (*8152*, GU139571, GU139576), Pico Verde lookout, San Miguel Island, Azores, Portugal, 800m, *Adams 8152-8153*; *J. macrocarpa*, (*7205*, GU139570, GU139575)15 km w of Tarifa on sand dunes, Spain, 36° 04.996'N, 5° 42.104' E, 30 m, *Adams 7205-7206*; *J. oxycedrus* (*9449*,

GU139567, GU139572) 4 km e of Forcalquier, France, 44° 04.06'N, 5° 59.19' E, 490 m, *Adams 9039*,1 km sw of Vila Nova de Foz Coa, Portugal, 41° 04.125'N, 7° 07.651' E, 360 m, *Adams 9449*. Voucher specimens are deposited at the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Phylogenetic trees Figure 3 shows a NJ tree based on nrDNA sequence
data.   A prominent feature of the tree is the clade of *J. cedrus, J.*

Figure 3. NJ tree based on nrDNA sequences. Numbers at the nodes
are bootstrap probabilities (1000 reps.).

macrocarpa and *J. oxycedrus* (Fig. 3). This is similar to the Bayesian tree (Fig. 2). *Juniperus brevifolia* is quite distinct compared to the *J. cedrus - macrocarpa - oxycedrus* clade. There is strong support (92%) for the clade of the Madeira junipers (putative *J. cedrus*).

The NT tree based on petN-psbM sequences has a congruent topology (Fig. 4), but the Madeira and Canary Island junipers are not resolved into clades as seen in the nrDNA data (Fig. 3).
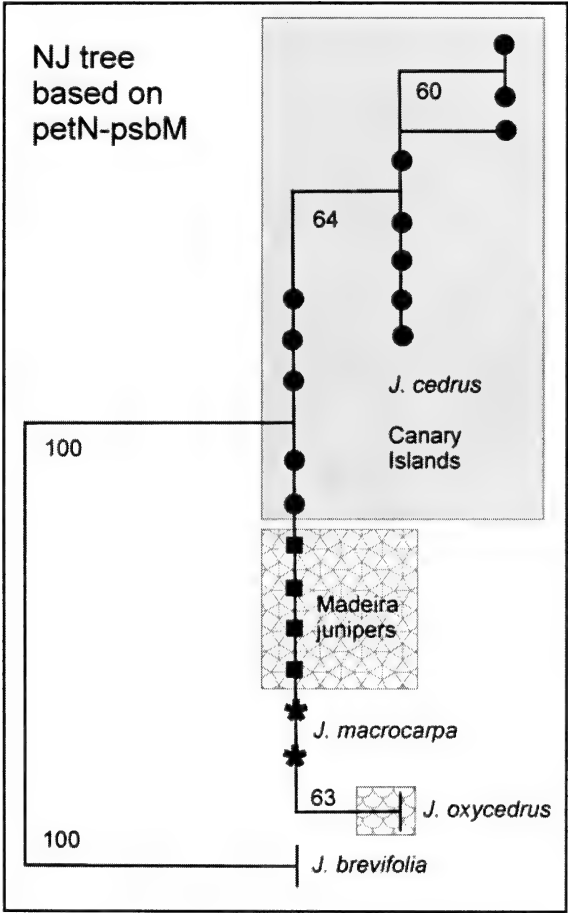
Figure 4. NJ tree based on petN-psbM sequences.

Combining the nrDNA and petN-psbM data, resulted in a NT tree (Fig. 5) that gives stronger support for the *J. cedrus*, Canary Islands and Madeira juniper clades. It should be noted that there is stronger support for the Madeira juniper clade than for the *J. macrocarpa* and *J. oxycedrus* clades. The evolution of the Madeira junipers is quite significant and correlates perfectly with leaf essential oil differentiation (Fig. 1).
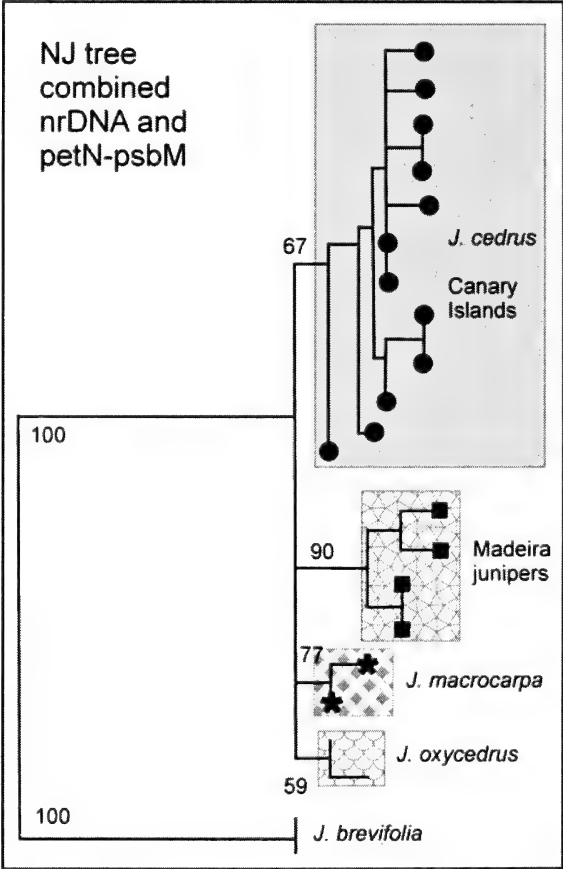
Figure 5. NJ tree based on combined nrDNA and petN-psbM data.

SNPs analyses   Figure 6 shows minimum spanning networks for nrDNA (left) and petN-psbM (right). The Madeira junipers are quite diverse in their nrDNA, but uniform in petN-psbM (Fig. 6). *Juniperus cedrus* from the Canary Islands are very uniform in both nrDNA and petN-psbM DNA.
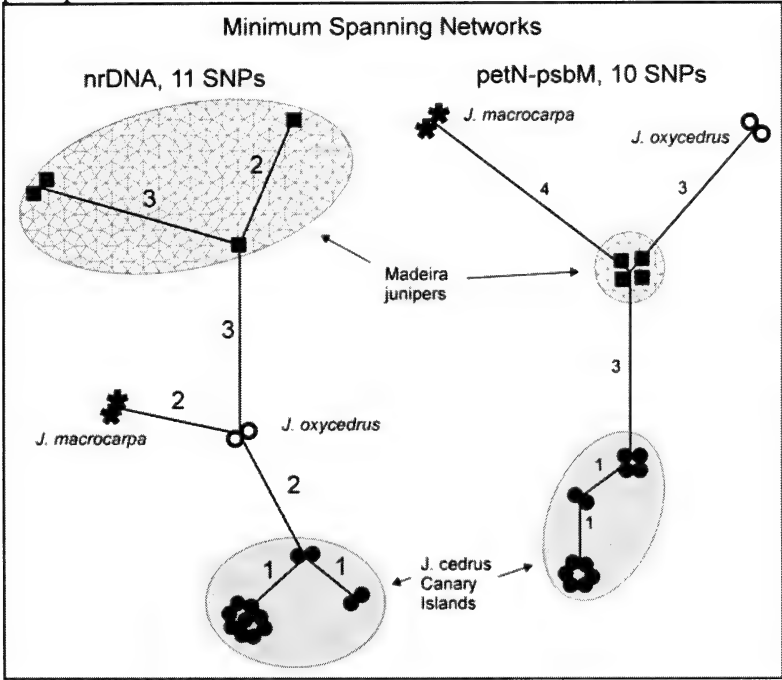
Figure 6. Minimum spanning networks for nrDNA and petN-psbM. Numbers next to the lines are the number of SNPs differences between nodes. Multiple symbols at nodes connote no differences among those individuals.

Combining SNPs from nrDNA and petN-psbM presents a clearer picture of the relationships (Fig. 7). Each of the groups differ by 6 or 7 SNPs. There seems to be considerable diversity in the Madeira junipers, but recall that this diversity is just in nrDNA, so it is
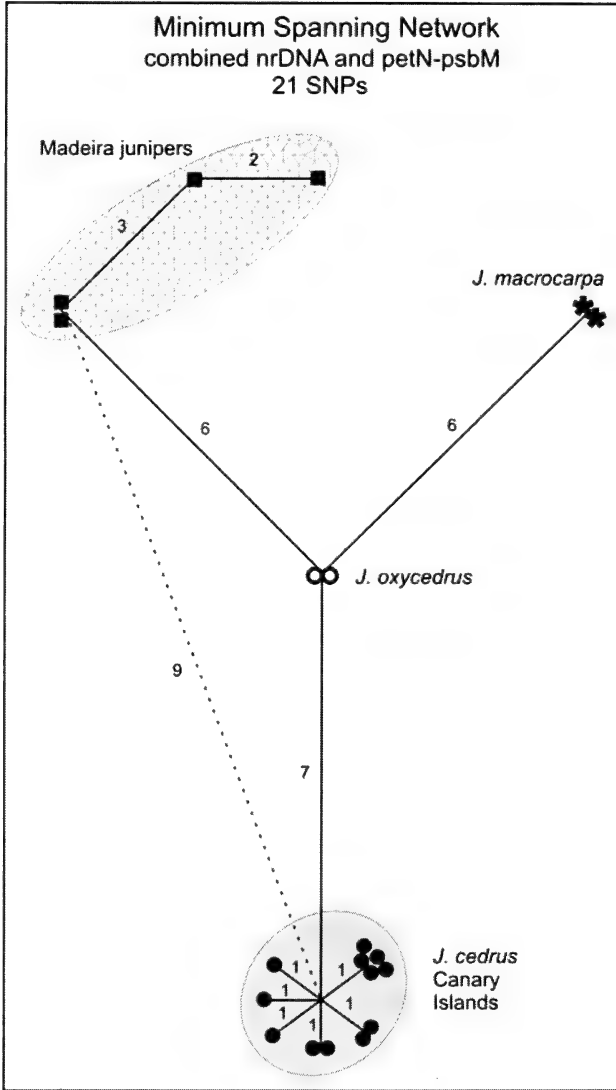
Figure 7. Minimum spanning network based on 21 SNPs from nrDNA and petN-psbM. The dotted line is the shortest link (9 SNPs) connecting the Madeira junipers with *J. cedrus*, Canary Islands.

possible that additional genetic data will not be as diverse. Notice (Fig. 7) that the nearest link between the Madeira junipers and the Canary Island *J. cedrus* is 9 SNPs.

Juniperus cedrus from Madeira has been treated as a subspecies (*J. cedrus* subsp. *maderensis* (Menezes) Rivas Mart. et al.). However, both the volatile leaf oil composition and the DNA sequence data support recognition of the Madeira juniper at the specific level:

⌐ ***Juniperus maderensis*** (Menezes) R. P Adams, **comb. et stat. nov.**
Basionym: *Juniperus oxycedrus* L. subsp. *maderensis* Menezes, Bull. Acad. Int. Geogr. Bot. 18 (No. 227-228): xii. 1908. Type: Madeira, Serra do Faial, Curral das Freiras, Portugal.

Distribution: Endemic to Madeira on the highest peaks in rocky areas. Nobrega collected it from Pico Ferreiro in 1988 and found 16 juniper plants on Pico das Torres in 1990. It is extremely rare and endangered in nature, but the seeds have been collected and it is now widely cultivated on Madeira.
Synonyms: *Juniperus grandifolius* Link, in Buch, Phys. Beschr. Canar. Ins.: 159. 1825. Farjon (2005) noted that this is a *nom. inval.* under Art. 34.1.
Juniperus cedrus Webb & Berthel. subsp. *maderensis* (Menezes) Rivas Mart. et al. Itinera Geobot. 15(2): 703. 2002.

Conservation: Although the species is rare and endangered in its native habitat, it is widely cultivated on Madeira Island. The greatest threat may be the introduction of related species (*J. cedrus* and *J. oxycedrus*) and possible hybridization with germplasm dilution effects. It would seem wise to prohibit the introduction of *J. cedrus* and *J. oxycedrus* into cultivation in Madeira and to continue to collect seeds and establish plants in cultivation on Madeira.

Because *Juniperus cedrus* does not grow on Madeira, it appears to be endemic to the Canary Islands. There, *J. cedrus* is composed of small, isolated populations. The diversity in its oils and DNA suggest that it is a mostly uniform taxon. Hence, due to the delicate status of conservation, it is urgent to restore old natural areas where *J. maderensis* previously grew on Madeira. It would seem prudent to

prohibit the introduction of *J. maderensis* from Madeira, or *J. oxycedrus* from Morocco, into cultivation in the Canary Islands due to possible hybridization with subsequent germplasm contamination of *J. cedrus*.

Table 1 shows some distinguishing features between *J. cedrus*, J. *maderensis* and *J. oxycedrus*.

Table 1. Distinguishing characteristics to separate *J. cedrus*, J. *maderensis* and *J. oxycedrus*.

	J. cedrus	J. maderensis	J. oxycedrus
Leaf tips	blunt to acute rarely mucronate tipped	usu. with a mucronate tip but not an elongated tip	elongated, mucronate tips
Stomatal bands	wider than midrib.	approx. as wide as midrib	approx. as wide as midrib
Leaf midrib	indistinct, usu. covered with bloom	distinct, green not covered with bloom	distinct, green not covered with bloom
Seed Cones (mature)	bright red, little or no bloom	brown-red, with bloom	brown-red to bright red, little bloom
Seed cones (mature)	larger than leaf length	~same as leaf length	smaller than leaf length

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LILIUM LANCIFOLIUM (LILIACEAE): NEW TO TEXAS

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ABSTRACT

Lilium lancifolium, the Tiger lily, is reported as new to Texas. *Phytologia 92(1): 56-58 (April, 2010).*

KEY WORDS: *Lilium*, Liliaceae, naturalized, Texas.

The Tiger lily (*Lilium lancifolium* Thunberg, formerly known as *L. tigrinum* Ker Gawler) is a native of eastern Asia (China) that is widely naturalized in eastern North America. In the Flora of North America, Skinner (2006) illustrates the distribution of the species from northeastern United States and adjacent Canada, west to North Dakota and Nebraska, and south to Missouri and Virginia, with isolated occurrences in Louisiana, Alabama, and North Carolina. The species is included in the Composite List of Weeds by Patterson et al. (1989).

Based upon the specimen cited below, the species may now be reported as naturalized in Texas.

Voucher specimen. **Texas. Morris Co**.: Park Road 17 near residence, Daingerfield State Park, 4 Jul 2009, *Ellen Buchanan, Craig Johnson, & Joan Thomas s.n.* (BAYLU).

Skinner (2002) describes the species as being easily diagnosed by its lanceolate and widely sessile alternating leaves that bear dark bulbils on the upper stem. These characteristics do not occur in the other two species of *Lilium* known to occur in east Texas.

The following key may be used to distinguish among the three species of *Lilium* that are known to occur in east Texas.

KEY TO THE SPECIES OF *LILIUM* IN EAST TEXAS

1. Perianth white (occasionally striated with green and red basally), 18–25 cm long; leaves linear, less than 0.6 cm wide
....*Lilium philippinense*
1. Perianth orange to orange-red, 5–10 cm long, spotted with purple to purple-brown; leaves lanceolate to
oblanceolate to narrowly obovate, 1.0–3.8 cm wide
....2
  2. Leaves whorled at midstem, sometimes alternate above, oblanceolate to narrowly obovate; axillary bulbils absent
....*Lilium michauxii*
  2. Leaves scattered to subopposite at midstem, lanceolate; axillary bulbils present at midstem ....*Lilium lancifolium*

Lilium michauxii Poir. The Carolina lily is a native species that is distributed from North Carolina south to Florida and west to Texas. In Texas the species is limited to rich woods, those characterized by *Fagus grandifolia*, *Quercus alba*, *Carya alba*, *Acer barbatum*, *A. leucoderme*, and *Ulmus rubra* in the southeastern part of the Pineywoods. This includes the following counties: Angelina, Cherokee, Hardin, Jasper, Nacogdoches, Newton, Rusk, Sabine, San Augustine, and Shelby counties.

Lilium philippinense Baker. This white flowered lily, native of the Philippine Islands, is reported by Skinner (2002) from one county in Kentucky and as "becoming well established in parts of Florida, especially near Tallahassee." Brown and Elsik (2002) reported the species as new to Texas under the very similar *Lilium longiflorum* Thunberg (Easter lily) from specimens collected in Newton and Tyler Counties. Brown recently indicated that the specimens were actually

Lilium philippinense (Diggs et al. 2006), who cited the distribution in the state as including Nacogdoches, Newton, and Tyler Counties. It is not clear if the Nacogdoches County record is supported by a specimen.

       Lilium philadelphicum L., the wood lily, is also known from Texas. The species is recorded from canyons of the Guadalupe Mountains of Culberson County in the Trans-Pecos of far west Texas (Correll and Johnston 1970). The species is also cited in Barton Warnock's (1974) Wildflowers of the Guadalupe Mountains and the Sand Dune Country of Texas as rare in South McKittrick Canyon of the Guadalupe Mountains. The species was not included as part of this study.

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GEOGRAPHIC VARIATION AND SYSTEMATICS OF *JUNIPERUS PHOENICEA* L. FROM MADEIRA AND THE CANARY ISLANDS: SNPS FROM nrDNA and petN-psbM

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ABSTRACT

SNPs from nrDNA and petN-psbM (cpDNA) were analyzed of *Juniperus phoenicea* from Madeira, Canary Islands, Morocco, and El Penon, Spain along with *J. p.* var. *turbinata* from the Tarifa sand dunes, Spain. The analysis of the 18 SNPs revealed that the Macaronesian and Moroccan plants are identical or nearly so to *J. p.* var. *turbinata* and quite differentiated from *J. phoenicea* var. *phoenicea*. In contrast, the leaf terpenoids showed that the oils of the Macaronesian plants are more similar to the Moroccan plants and not as similar to *J. phoenicea* or *J. p.* var. *turbinata*. At present, it seems prudent to treat the Madeira and Canary Island plants as *J. p.* var. *turbinata*. *Phytologia 92(1):59-67 (April, 2010)*.

KEY WORDS: *Juniperus phoenicea, J. p.* var. *turbinata,* Cupressaceae, Madeira Island, Canary Islands, leaf essential oils, SNPs, nrDNA, petN-psbM.

Juniperus phoenicea L. is a very variable species of the Mediterranean region (Adams, 2008). The Canary Islands red-fruited, scale-leaf juniper was described as *J. canariensis* Guyot & Mathou,

(Trav. Lab. Forest. Toulouse T. 1 [3, 2]: 7. 1942) and later treated as *J. turbinata* Guss. subsp. *canariensis* (Guyot & Mathou) Rivas-Martinez et al. (Itinera Geobot. 7: 511. 1933).   Farjon (2005) treated *J. canariensis* as a synonym of *J. phoenicea* var. *phoenicea* and recognized *J. phoenicea* var. *turbinata* (Guss.) Nyman as "restricted to littoral maritime habitats on rocks or sand dunes." in the Mediterranean in France, Spain, Portugal, Greece, Italy, Morocco, and Tunisia." Farjon (2005) treated the Macaronesian plants as *J. p.* var. *phoenicea*. Adams et al. (1996, 2002) showed that *J. p.* var. *turbinata* and *J. p.* subsp. *eu-mediterranea* Lebr. & Thiv. were the same taxon.   Adams (2008, map p. 243,) treated the Madeira and the Canary Islands *J. phoenicea* as "var. *turbinata* ?"

RAPDs analysis (Adams et al., 2006) of *J. phoenicea* from sand and rock areas in Morocco, plants from Tenerife, Canary Islands and var. *turbinata*, Tarifa sand dunes, Spain showed (Fig. 1) that var. *phoenicea* (El Penon, Spain) was well resolved from the Morocco, Tenerife and var. *turbinata* populations. The Tenerife population accounted for about 14%
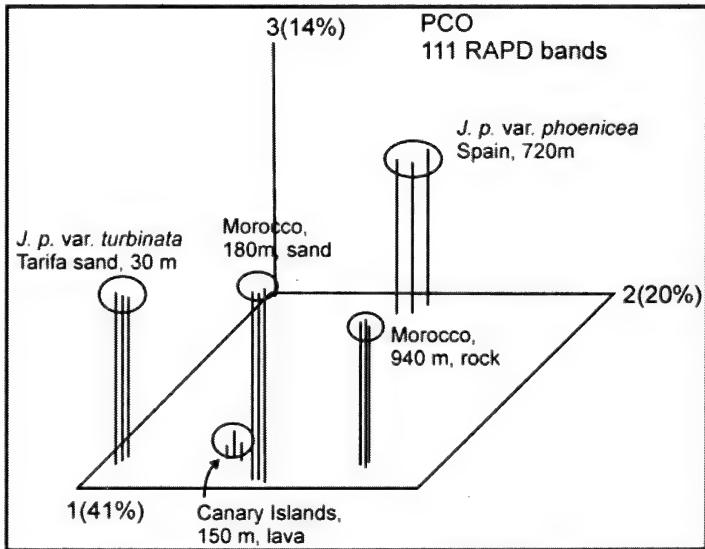
Figure 1. PCO ordination of *J. phoenicea* populations based on 111 RAPD bands. From Adams et al. (2006).

of the variance (Fig. 1). Although, the Canary Island plants are loosely associated with var. *turbinata*, they generally have large, round berries (seed cones), not turbinate-shaped.

Adams et al. (2009) reported on the volatile leaf oil compositions of populations of *J. phoenicea* from several islands in the Canarian archipelago and Madeira, and compared these oils with *J. p.* var. *phoenicea* (Iberian Peninsula, Spain) and var. *turbinata* (Tarifa sand dunes, Iberian Peninsula, Spain) oils. They found that the oils varied a little among the islands but Madeira, La Palma, Tenerife, and La Gomera do form a group (Fig. 2). However, the oils are also similar to Morocco (0.70) and less similar to var. *phoenicea* (Spain) and var. *turbinata* (Spain) (Fig. 2).
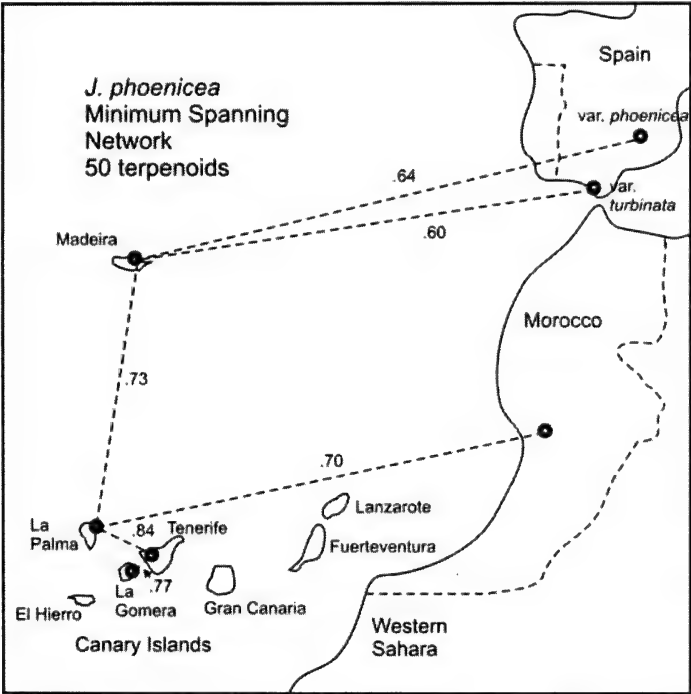
Figure 2. Minimum spanning network based on 50 terpenoids. From Adams et al. (2009).

The purpose of this paper is to report on analyses of SNPs from nrDNA and petN-psbM (cp DNA) and to compare the DNA data with the previous terpenoid analysis.

MATERIALS AND METHODS

Plant material (GenBank Accession: ITS, petN-psbM) - *J. phoenicea* Madeira Island: 1 km NE of Santana, 32° 48.822'N, 16° 52.627'W, ca 100 m, *R. P. Adams 11502, 11503,* cultivated at Botanic Garden in Funchal, putatively ex Madeira Island, 32° 39.08'N, 16° 47.14'W, ca 200 m, *R. P. Adams 11504*; Canary Islands: Tenerife, volcanic rock, ca. 150 m, *R. P. Adams 8147-8149*, La Palma Island, Santa Lucia, loose volcanic pumice, 28° 44.250'N, 17° 44.198'W, 283 m, *R. P. Adams 11514-11516*, La Gomera Island, volcanic rock, 28° 11.358'N, 17° 12.320'W, 370 m, *R. P. Adams 11528(GU197868, GU197871)-115230;* Spain, limestone soil, 25 km e. Guadahortuna, 720 m, El Penon, *R. P. Adams, 7077(GU197869, GU197873)-7079*; Morocco, red clay, 20 km sse Marrakech, 31° 21.033'N, 07° 45.893'W, 940 m, *R. P. Adams 9408-9410;* Iberian Peninsula, Spain, *J. phoenicea* var. *turbinata*: Tarifa sand dunes, 15 km w. of Tarifa, 30 m, 36° 04.996'N, 5° 42.104' W, *R. P. Adams, 7202(GU197870, GU197873)-7204*. Voucher specimens are deposited at the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Sequencing nrDNA revealed 15 nucleotide mutational events that included 3 indels and 3 mutations that occurred in only one individual among the taxa. The 3 single nucleotide changes were discarded from the SNPs leaving 12 nrDNA characters.

Sequencing petN-psbM revealed 8 nucleotide mutational events that included a 19 bp deletion in one of the plants from El Penon, Spain and 1 mutation that occurred in only one individual among the taxa. Discarding these 2 singular events provided 6 petN-psbM characters.

PCO using the 18 SNPs resulted in eigenroots that accounted for 58% (axis 1), 24% (axis 2) and 9% (axis 3) of the variance among the individuals. Ordination shows that the major trend was to separate *J. phoenicea* var. *phoenicea* (El Penon, Spain) from all other individuals (Fig. 3). The two natural trees from Madeira Island and 2 trees from La Gomera appear to share the same cpDNA petN-psbM in that they differ by 3 SNPs from the other Canary Island trees. It is interesting that var. *turbinata* (Tarifa sand dunes, Spain) is identical

with one Canary Islands tree (from Tenerife) and differs by only one SNP from most other Canary Island plants (Fig. 3). The *J. phoenicea* plant cultivated at Botanic Garden in Funchal (11504), putatively ex Madeira Island, differs by 6 SNPs from *J. phoenicea*, naturally growing on Madeira (Fig. 3). It seems likely that the cultivated plant actually came from the Canary Islands. Note that the 2 plants nearest to the 2 natural Madeira plants (Fig. 3) are from La Gomera. The 3 SNPs that separate these La Gomera plants from the bulk of the Canary Island plants are in the cpDNA region. So it may be that long distance dispersal between La Gomera and Madeira has caused this result.

Figure 3. PCO based on 12 nrDNA and 6 petN-psbM SNPs. The numbers on the dashed lines are the number of SNPs in the minimum linkage network.

A minimum spanning network superimposed on a geographic map gives a little different perspective (Fig. 4). The large difference between *J. phoenicea* var. *phoenicea* (Spain) and *J. phoenicea* of the Canary Islands is clearly seen. The small or no differences between plants very widely separated is also apparent (cf. Morocco - cult. Madeira plant; Madeira (natural) - La Gomera; Tenerife plant - var. *turbinata*, Spain).
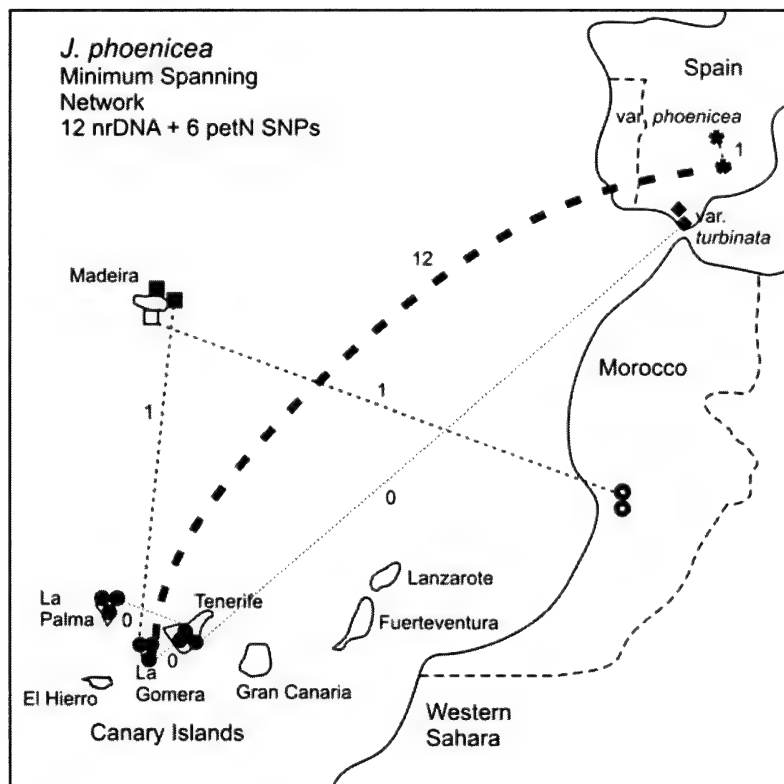
Figure 4. Minimum spanning network based on 12 nrDNA plus 6 petN-psbM SNPs. The numbers next the dashed lines are the number of SNPs separating the OTUs. Not all links are shown due to congestion on the small islands.

Both the terpenoids and SNPs clearly show that the Canary Island and Madeira *J. phoenicea* is quite different from *J. phoenicea* var. *phoenicea* (El Penon, Spain). Both data sets show a close affinity of plants from the Canary Island and Madeira to plants from Morocco. However, there is a marked difference in how the data depicts the relationship of *J. phoenicea* var. *turbinata* to Madeira and Canary Island junipers. The terpenoid data showed var. *turbinata* to be the least similar (0.60) to any island junipers. In contrast, the SNPs data showed var. *turbinata* to be indistinguishable from a tree on Tenerife and almost identical to most plants from the Canary Islands, Madeira and Morocco. Although the terpenoid data supports the recognition of *J. p.* subsp. *canariensis*, the SNPs analysis does not support it and favors the recognition of the Canary Islands and Madeira *J phoenicea* trees as *J. p.* var. *turbinata*.

It seems probable that evolution is proceeding at different rates in the terpenoids than in the nrDNA and petN-psbM DNA. It may be that the terpenoids are under stronger adaptive selection pressure and reflect the differences in the survival ecology on the Tarifa sand dunes of Spain, versus the volcanic islands of Madeira and the Canary Islands. Certainly these habitats differ considerably in the environment, herbivores, insects and diseases faced by the junipers.

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DISCOVERY AND SNPS ANALYSES OF POPULATIONS OF *JUNIPERUS MARITIMA* IN THE OLYMPIC PENINSULA, A PLEISTOCENE REFUGIUM?

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ABSTRACT

Trees from two populations of *Juniperus* commonly identified as *J. scopulorum* growing on the northeast side of the Olympic mountains were analyzed by SNPs from nrDNA and petN-psbM (cpDNA) and compared with *J. scopulorum* (UT), *J. virginiana* (TN) and *J. maritima* from Puget Sound. The trees of the Olympic Mountains were found to be *J. maritima*. The habitat of these newly investigated populations of *J. maritima* are atypical in that they occupy high elevation sites (600 - 1700 m), whereas most previous *J. maritima* sites are seaside. The Deer Park population (1700 m) plants are Krumholz due to the high winds and snowpack conditions. The Cordilleran ice sheet is mapped and the Olympic peninsula is proposed as the site of a Pleistocene refugium for *J. maritima*. *Phytologia 92(1): 68-81 (April, 2010)*.

Juniperus maritima R. P. Adams, the seaside juniper, is a rare species found on the seashores in the Puget Sound - Strait of Georgia area (Adams, 2007, 2008). Examination of herbarium specimens revealed that *Juniperus* trees identified as *J. scopulorum* Sarg. have been reported from the dry side (northeast) of the Olympic Mountains on the Olympic Peninsula of Washington. The female cones on these specimens had insect damage and the exserted seeds typical of *J. maritima*. Specimens were analyzed from 2-4 m trees at 3 o'clock ridge (912 - 960 m) and Krumholz shrubs (25-30 cm tall) at Deer Park (1670 m) on the northeast side of the Olympics.

The purpose of this paper is to report on the analyses of SNPs from nrDNA and petN-psbM (cpDNA) for these putative *J. maritima* plants in comparison with *J. maritima* from seaside locations and with typical *J. scopulorum* from Utah.

MATERIALS AND METHODS

Specimens used in this study: *J. maritima, Adams 11056-58*, Brentwood Bay, Vancouver Isl., BC, *Adams 11061-63*, Cowichan Bay, Vancouver Isl., BC, *Adams 11064*, Yellow Point, Vancouver Isl., BC, *Adams 11065-66*, Lesqueti Isl., BC, *Adams 11067-68*, Friday Harbor, San Juan Isl., WA, *Adams 11075*, Whidbey Isl., Cranberry L., WA, *Adams 11076*, Washington Park, Fidalgo Island, Anacortes, WA, *Adams 11077-78*, Skagit Isl., WA; *Adams 11999-12001*, 912-960m, 3 o'clock ridge, Olympic National Forest, WA, *Adams 12002-12004*, 1670 m, Dear Park, Olympic National Park, WA, *J. virginiana, Adams 10230-10232*, Knoxville, TN; *J. scopulorum, Adams 10895- 10897*, Kamas, UT. Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

PCR amplification   ITS (nrDNA), petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl$_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.   The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.).   In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit.   The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing.   Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.).   Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975).   Minimum spanning networks were constructed (Adams et al. 2009).   Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Analysis of the nrDNA (ITS) sequences revealed 36 mutational events of which 19 mutations were found in only one individual and removed from the data. This resulted in 17 SNPs among *J. maritima*, *J. scopulorum*, *J. virginiana*, and the junipers from the Olympics for analysis.   A minimum spanning network based on these 17 SNPs from nrDNA is shown in figure 1.   The individuals are arranged in three groups: *J. virginiana*, Knoxville, TN, no variation; *J. scopulorum*, Kamas, UT with some variation; and the central group consisting of *J. maritima* from Puget Sound - Strait of Georgia and the

plants from the Olympic mountains with a few SNPs separating individuals, but no pattern among them. Individuals from the Olympic mountains are intermixed with *J. maritima* from seaside locations.

Analysis of petN-psbM (cpDNA) sequences yielded 52 mutational events. Fourteen mutations were found in only one individual and removed from the data leaving 38 SNPs among *J. maritima*, *J. scopulorum*, *J. virginiana*, and the junipers from the Olympics for analysis. A minimum spanning network based on these 38 SNPs from petN-psbM sequences is shown in figure 2.

Two plants (*11075*, Whidbey Isl., Cranberry L., WA, *Adams 11076*, Washington Park, Anacortes, WA) share a haplotype that differs by 17 SNPs from other *J. maritima* plants. In contrast with the nrDNA (Fig. 1), only two groups are present: *J. scopulorum* (bottom) and a diverse group in the center consisting of *J. virginiana*, *J. maritima* from seaside locations and the Olympic mountains plants. Adams (2007) reported that cpDNA did not fully separate *J. maritima* and *J. virginiana* plants. It is interesting that the Olympics plants do form distinct groups (Fig. 3). However, the 3 o'clock ridge (960m) plants are intermixed with the Deer Park (1670 m) Krumholz plants (Fig. 3).

Removing the 2 divergent haplotypes of *J. maritima* and combining the sequences (nrDNA plus petN-psbM) resulted in 34 SNPs. A minimum spanning network based on these 34 SNPs (Fig. 3) shows three groups: *J. virginiana*, *J. scopulorum* and *J. maritima* from seaside locations and the Olympic Mountains plants. It is interesting that the Olympic Mountains plants are in the center of the network, between *J. scopulorum, J. virginiana* and *J. maritima* seaside plants. There is considerable variation among the *J. maritima* plants, but it is clear that the Olympic Mountains plants are part of *J. maritima*, and not *J. scopulorum* nor *J. virginiana*.

The presently understood distribution of *J. maritima* is shown in figure 4. It seems likely that additional populations will be found on islands and the seashore of the Strait of Georgia. It appears that *J.*
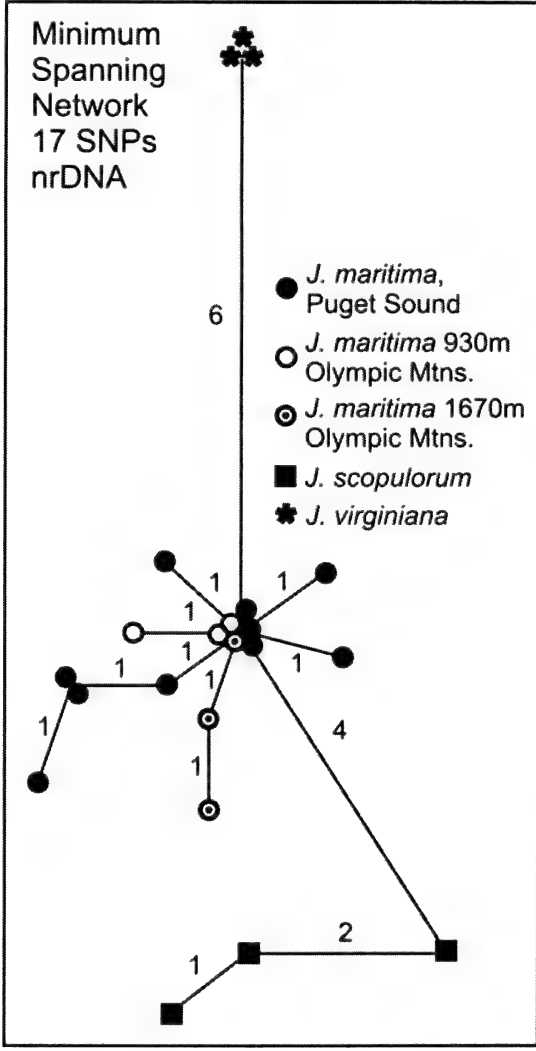
Figure 1. Minimum spanning network based on 17 SNPs from nrDNA. Notice the central group composed of seaside *J. maritima* from Puget Sound and the putative *J. maritima* from the Olympic mountains. Numbers next to the lines are the number of SNPs in that link.

Figure 2. Minimum spanning network based on 38 SNPs from petN-psbM sequences. Note that two *J. maritima* plants (Fidalgo and Whidbey Islands) share a haplotype that differs by 17 SNPs from other *J. maritima* plants.
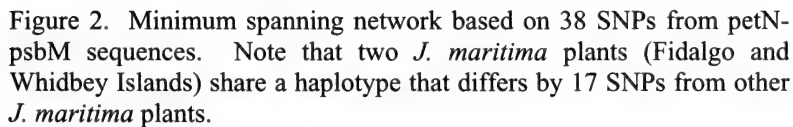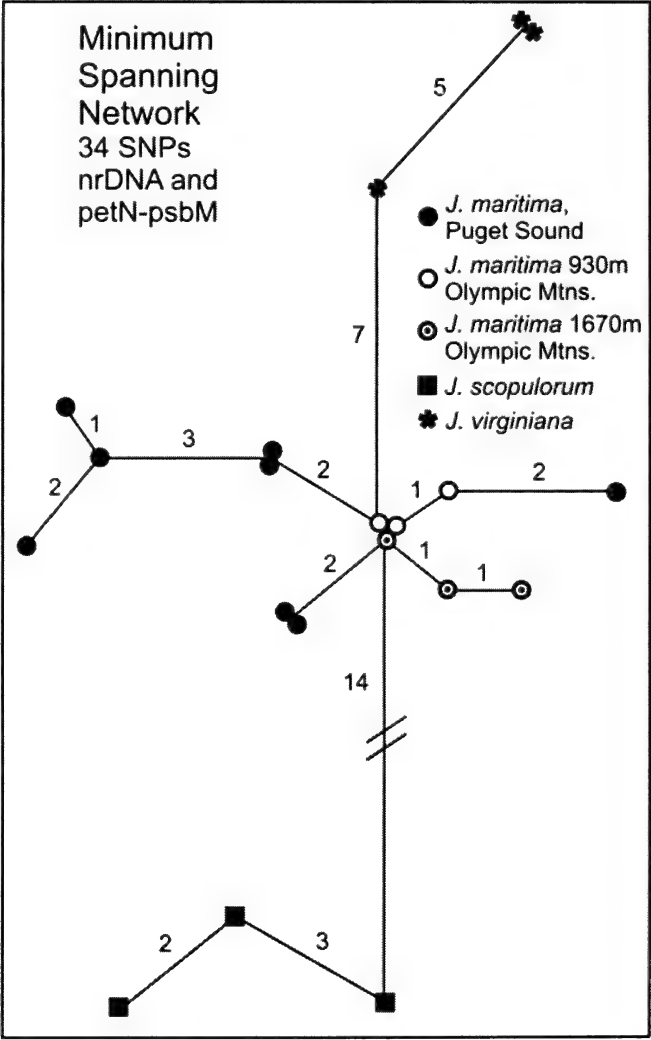
Figure 3. Minimum spanning network based on 34 SNPs from both nrDNA and petN-psbM.
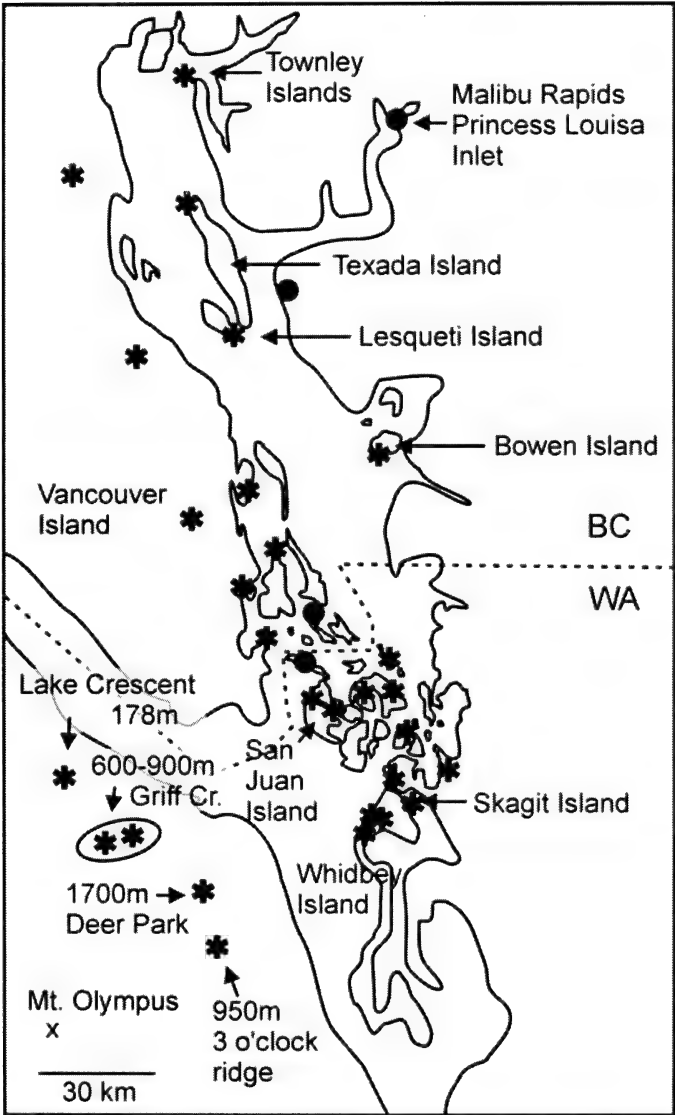
Figure 4. Distribution of *Juniperus maritima* based on Adams field collections, herbarium specimens from V, WS, and WTU (asterisks), and sight observations by Tom Fairhall (closed circles).

maritima's northernmost populations are on the Townley Islands and at Malibu Rapids at the Princess Louisa Inlet (Fig. 4). *Juniperus maritima* trees in the Olympics Mountains below 960 m are shorter than the seaside trees in Puget Sound - Strait of Georgia (Fig. 5).   The population of *J. maritima* at Deer Park is Krumholz (Fig. 6) and only about 25-30 cm tall.

Fig. 5. *Juniperus maritima* as a tree on 3 o'clock ridge, 960m, Olympic National Forest, mixed with Pseudotsuga menziesii.

         Adams (1983), using terpenoid data, considered the Puget Sound junipers to be divergent populations of *J. scopulorum*.   He examined Pleistocene refugia and recolonization and hypothesized that the Puget Sound populations prior to extinction moved south of the Olympic peninsula, then recolonized Puget Sound when the Cordilleran ice sheet retreated (Figs. 9, 10; Adams, 1983).   All of the present seaside populations of *J. maritima* in Puget Sound and the Strait of Georgia were under the Cordilleran ice sheet (Fig. 7).   The present-day high elevation populations in the Olympic Mts. were likely at the very edge or under alpine glaciers (Fig. 4, Buckingham et al. 1995).

However, most of the Olympic Mountains were thought to be ice free (Fig. 7), except for glaciers at higher elevations.

Fig. 6. *Juniperus maritima* as a Krumholz shrub at 1670 m at Deer Park, Olympic National Park.

Buckingham et al. (1995) suggest a strip of land along the west coast of the Olympic peninsula as wide as 50 km was exposed during the Wisconsin to provide additional plant refugia. They considered it unlikely that *J. scopulorum* (*J. maritima*) retreated to northwestern Oregon, but rather, found a refugium in the Olympic Mountains itself. Lacking any fossil evidence, one can only conjecture that *J. maritima* was in fact a 'seaside' juniper in the Strait of Georgia and Puget Sound before the Pleistocene.

Although it has been widely held view that species retreated from glacial ice into warmer refugia, Opgenoorth et al. (2010) present DNA evidence that populations of *Juniperus* on the Tibetan plateau may have persisted in cryptic or micro-refugia within a few hundred

meters of glacial ice during the Pleistocene. The micro-refugia theory is controversial and additional cases need to be examined to validate it. Nevertheless, it does lead to an interesting hypothesis that *J. maritima* might have survived near the front edge of the Puget Lobe of the Cordilleran ice field. With the discovery of *J. maritima* at high elevation sites (this paper), it does seem possible that *J. maritima* survived the Pleistocene in the Olympic peninsula. It may be that before the Pleistocene, *J. maritima* was a montane species and that it merely spread to open habitat on rocky seashores in the Strait of Georgia and Puget Sound during the Hypsithermal (10,000 - 6,000 ybp, see Buckingham et al. 1995). The DNA data presented in this study did not show much differentiation between the seaside populations and the montane populations of the Olympic mountains. Additional analyses of more sensitive markers may reveal differentiation, but at present it appears that the seaside populations of *J. maritima* are of very recent origin with little differences from the montane (ancestral) populations.

Figure 7. Cordilleran ice sheet limits. Cross-hatched areas around Mt. Olympus are hypothetical glaciers. Based on Booth et al. (2004) and Buckingham, et al. (1995).

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SCREENING FOR LOW-COPY NUCLEAR GENE REGIONS IN THE CUPRESSACEAE

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ABSTRACT

Thirty two low-copy nuclear (LCN) gene regions from Steele et al. (2008) were amplified using *Hesperocyparis goveniana* (Cupressaceae) DNA. Ten of these gave successfully amplified products and these were sequenced. Four of these LCNs yielded clean sequences. Of the 4 LCNs, one proved to be a mitochondrion gene region with no variation between *Hesperocyparis* and *Juniperus*;. The second LCN, tentatively identified as Actin-11, gave 497 bp of clean sequence then appeared to be multi-copy. The third LCN, tentatively identified as part of 3-carene synthase, gave 924 bp of clean sequence in *Hesperocyparis* but appeared to be multi-copy in *Juniperus*. The fourth LCN, possibly heat shock protein 90.1, yielded 946 bp of good sequence data for *H. goveniana, J. scopulorum* and *J. virginiana* and appears to offer promise for utilization in Cupressaceae taxonomy. *Phytologia 92(1): 82-91 (April, 2010).*

KEY WORDS: Low-copy nuclear genes, Cupressaceae, *Juniperus*, *Hesperocyparis*.

The major nuclear gene region utilized for sequencing in plants is nrDNA (nuclear ribosomal DNA) that has apparently undergone concerted evolution (Baldwin et al. 1995). It is also the major nuclear region sequenced in the *Cupressaceae*. However, nrDNA (ITS) is sometimes not sufficiently variable to distinguish closely related species and varieties (Adams and Bartel, 2009). 4-coumarate CoA ligase (4CL) and abscisic acid-insensitive 3 (ABI3) are two nuclear genes that have recently been employed in *Juniperus* (Adams, 2009) and the Cupressaceae (Adams, Bartel and Price, 2009),

but neither gene region is sufficiently variable to resolve some closely related species or varieties.

Non-coding areas of chloroplast DNA (cpDNA) have been widely utilized but as Steele et al. (2008) discuss, cpDNA is of limited use in examining hybridization in the Cupressaceae with uni-parental inheritance (paternal) and chloroplast capture. Adams, Schwarzbach and Morris (2008) found only 1 bp variation in cp trnC-trnD among the 8 Caribbean *Juniperus* taxa. Most Caribbean junipers had no variation in trnC-trnD.

Recently, Steele, et al. (2008) examined 141 low-copy nuclear (LCN) gene regions in two families of rosids. From these 141 LCNs, they list 32 as most promising.

The purpose of this paper is to report on analyses of these 32 low-copy number gene regions as applied to *Juniperus* and *Hesperocyparis* (Cupressaceae) for possible phylogenetic utilization.

MATERIALS AND METHODS

Genomic DNA utilized: Juniperus ashei: Adams 6746-48, Waco, TX; *J. blancoi, Adams 6849-51*, Durango, MX; *J. scopulorum, Adams 10895-10897*, Kamas, UT; *J. virginiana, Adams 6753-6755,* Hewitt, TX; H*esperocyparis goveniana, Adams and Bartel 9350-9351*, Monterey, CA. All vouchers are stored at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR primers (Table 1) were synthesized by Integrated DNA Technologies (San Diego, CA).

PCR amplification Various LCNs were amplified as shown in Table 1. In general, amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl

2x buffer E (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) and 1.8 μM of each primer.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). When possible, the band(s) was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/).

RESULTS AND DISCUSSION

Table 1 shows the forward and reverse primers for 32 most promising LCN gene regions from Steele et al. (2008).

Table 1. Primers for the 32 most promising gene regions of 141 screened by Steele et al. (2008).

Steele LCN primer pairs	sequence (5'-3'	For/Rev
1F	GGTTTAGTGAAAATATCAGC	For
1R	TATGGTTTGAAACAAGCACCT	Rev
5F	ATCCAGAAGGAGTCCACCCTTCA	For
5R	TCCTTCTGGATGTTGTAGTCGG	Rev
6F	GATGGACAGGTGATCACCATTGG	For
6R	TAGAAGCACTTCCTGTGGA	Rev
13F	CGTCACCGTCTGCGAGATCAAC	For
13R	TACCTTGAGAAACATCCC	Rev
15F	ACGGACAAGAGCAAGCTCGATG	For
15R	TTGTAGTCTTCCTTGTTCTCAG	Rev
30F	GCTAGCCATGTCCTTGAGGT	For
30R	CAGATTTTGGGCTTGCCAAAGAT	Rev
31F	CAGATGAGCTGATCAAGACGGCC	For

31R	ACCTTTGGGCTGTCGGAGC	Rev
46F	ATGCAGATCTTCGTGAAGAC	For
46R	TCCTTCTGGATGTTGTAGTCGG	Rev
50F	TCTGAGTTCATCAGCTACCC	For
50R	GTTGTAGTCTTCCTTGTTCTCAG	Rev
51F	CCTAGCTTGATGACACCAC	For
51R	CTTGGACGTATCATGAAT	Rev
56F	GTCCTTCGCTTCAGGGCCTG	For
56R	TTTCATGATAGCAAGGTGCTCCC	Rev
57F	AAGGAGGTTTCTCATGAGTGG	For
57R	GTCTTCTTGCTCGACATGT	Rev
58F	AACATCTCCTGGATTGAGGT	For
58R	GACAATGAGGCTCTCTAC	Rev
61F	ATGGGACAAAAAGATGCTTA	For
61R	TAGAAGCACTTCCTGTGGA	Rev
64F	AACCGTCCCAACAGCATTGA	For
64R	GCACCACTGAAGCCTTGGGT	Rev
65F	TTTGTCAGTACAGTGGATCC	For
65R	CTTTGTGGGAATTGGACAT	Rev
68F	GAGTTCATGACATCAATGG	For
68R	ACCCTGGAGATCGGCATGG	Rev
69F	CATTGAGGAGAACAATGCAGG	For
69R	TGCTCAGGAGCACCTTTG	Rev
73F	CGGGTTCGAGTCCCGGCAACGG	For
73R	TGGAACCACTTCATGGCAT	Rev
81F	CGAGACCACCAAGTACTACTGC	For
81R	TCATCTTAACCATACCAG	Rev
82F	CAAGAGGTCCTTCAAGTACGC	For
82R	TCATCTTAACCATACCAG	Rev
85F	CTGTGACAATGGAACCGGAATGG	For
85R	TAGAAGCACTTCCTGTGGA	Rev
86F	ATGGTTGAGTACTTTGGTGAGCAG	For
86R	GCCCAGTCCAAGTAGAAAG	Rev
87F	GTGATCACTACATCAACAATAGC	For
87R	GTAACAGAACCATAGATCC	Rev
90F	CAGGTTCTCATTTGGGATGT	For
90R	CCTGAACACAAAGAACAGCAG	Rev

91F	GTGGTCTTGTCCTCAGCAGA	For
91R	TGCGTATCATCAACGAGC	Rev
94F	GAGAGCAGCGACACCATCG	For
94R	CCAGTGAGTGTCTTCAC	Rev
96F	TGGTGTGATACAAAATCAGT	For
96R	TCAGGGCTAATATCAGTA	Rev
125F	GAGCCATACAAGGGTATT	For
125R	GACCACGGTAGACAATGA	Rev
126F	TGCGCTATTTACCAGTGAGTGG	For
126R	AAAGGGGATGTTTTGTTAA	Rev
129F	TATGCCAGTGGTCGTACAAC	For
129R	TTAGAAGCATTTCCTGT	Rev
133F	ATGAAATCATTCTTGAGGAC	For
133R	CTACGCCAACAAGGTCGGCC	Rev

Each of these 32 primer pairs were utilized with *H. goveniana* DNA to determine amplification at 3 annealing temperatures (Table 2). Three of the LCN gene primers gave primer dimers (31, 81, 83) at all annealing temperatures (Table 2). Eight of the LCN gene primers gave only faint bands or smears (13, 50, 58, 65, 85, 96, 126, 129). It is worthwhile to recall that Steele et al. (2008, Appendix A) showed that most of their 141 LCN gene primers failed to amplify.

A few of the primers gave bright bands at all 3 annealing temperatures (1, 5), but generally the brightest bands were present at only one (sometimes two) of the three annealing temperatures (Table 2). Several of the LCN gene primers gave bright bands but of small size: 5 (250bp), 15 (450bp), 46 (460bp), 64 (500bp), 68 (300, 400 bp), 73 (360 bp), 90 (300bp), 91 (300bp), 125 (500bp) and 123 (400bp). Most of these were eliminated from further consideration for sequencing. It would be interesting to compare the sizes of these bands with those in the rosids by Steele et al. (2008).

Some of the LCN gene primers gave a bright band that upon closer examination or by running the gel longer turned out to be twin bands (51, 64, 68, 69, Table 2).

Table 2. Amplification results using annealing temperatures of 45ºC, 50ºC and 55ºC. Amplified products (in bold face) were prepped and sequenced. brt = bright, med = medium, and f = faint band. Band sizes were estimated using pGEM markers on a 1.6% agarose gel.

LCN	45ºC	50ºC	55ºC
1	**brt 950, 200 bp**	same as 45ºC	same as 45ºC
5	brt 250+f 350 bp	same as 45C	same as 45C
6	brt 1500+700bp	**brt 1500+700bp**	faint bands
13	faint bands	faint bands	faint bands
15	**brt 450 bp**	same as 45C	faint 400bp
30	**brt 500bp**	brt 500bp	nothing
31	primer dimer	primer dimer	primer dimer
46	brt 460+ 220 bp	**brt 460**+220bp	brt 460+ 220bp
50	smear	1 faint band	1 faint band
51	brt 500bp (2bands!)	2 faint bands	2 faint bands
56	brt 900bp	brt 900bp	**brt 900bp**
57	brt 200bp	brt 200,faint 950bp	**brt 950bp**
58	smear	nothing	nothing
61	4 bright bands	1 faint band	nothing
64	brt 500bp (2 bands)	3 brt 600-400bp	1 faint band
65	3 faint bands	nothing	nothing
68	2 brt bands, 300-400	2 faint bands	nothing
69	2 brt 600, 1 f 800bp	**brt 800bp ,2 f**	nothing
73	brt 360, f 500bp	brt 360, f 500bp	faint 360 bp
81	primer dimer	primer dimer	primer dimer
82	primer dimer	primer dimer	primer dimer
85	faint 360bp	faint 360bp	v faint 360bp
86	smear	f 600, 3brt 300bp	smear
87	**brt 650bp + smear**	faint 650bp	v faint bands
90	brt 300, f500, 200bp	f500,f300,f200bp	faint smear
91	brt 300bp + smear	f 300bp	nothing
94	**brt 500 + brt 300bp**	brt 500+m300bp	f 500+b300bp
96	smear	smear	smear
125	brt 500+m 700bp	faint	faint
126	smear	smear	smear
129	nothing	nothing	nothing
133	f800,f600,brt 400bp	m800,m600bp	faint

        Based on the screening (Table 2), 10 LCN gene regions were selected for prep and sequencing for *H. goveniana.* These are highlighted in Table 2) and are LCN gene regions 1 (950 bp, 45°C), 6 (600 bp, 50°C), 15 (450 bp, 45°C), 30 (500 bp, 45°C), 46 (460 bp, 50°C), 56 (900 bp, 55°C), 57 (950 bp, 55°C), 69 (800 bp, 50°C), 87 (650 bp, 45°C) and 94 (500 bp, 45°C). Of course, some of these are marginal in size as it may not be practical (cost-effective) to sequence only 450 bp if one can find LCN gene regions that are 900 - 1000 bp. But it was felt that determining how many of the 10 regions would yield clean sequence data was sufficient reason to include these smaller regions in the sequencing.

        Sequencing the 10 most promising 32 LCN gene regions (Table 2) gave 4 LCNs with partial or complete sequences (Table 3, 1, 6, 56, 57). LCNs 15, 30, 69, 87 and 94 appeared to be multi-copy in the *H. goveniana* sequences. For LCN 46, the prep band yield was too low to sequence.

        Four of the 10 LCN gene regions seemed worthy of additional study:

LCN 1.   Sequencing in *H. goveniana* gave 924 bp of nice, clean sequence but when amplifications were attempted in *Juniperus ashei, J. blancoi, J. scopulorum* and *J. virginiana* 2 bands were produced that could only be partially separated by electrophoresis to 140 min. at 70 V on a 1.6% agarose gel.   The highest GenBank match for the *H. goveniana* sequence was to the *Picea glauca* FJ609175, putative 3-carene synthase gene (Hamberger, et al. 2009).   The terpene synthase genes are known to be in families (Trapp and Croteau, 2001; Hamberger, et al. 2009) and appear to have arisen by gene duplication. It may be very difficult to obtain single copy genes in this family. However, by designing new, interior primers to the *H. goveniana* sequence, one might be able to obtain specific primers that would amplify single copies in *Juniperus* and other Cupressaceae species.

Table 3. Sequencing of the 10 most promising Steele LCN gene regions.

Steele LCN	Size	Sequencing result/ gene
1	924 bp	clean in *H. goveniana*, multi-copy in *Juniperus* 3-carene synthase? *Picea glauca* FJ069175
6	~1500 bp	497bp clean seq. in *H. goveniana.* then messy, multi -copy? Actin-11, *A. thaliana* U27981
	~700 bp	multi-copy, messy seq. in *H. goveniana.*
15	~450 bp	multi-copy in *H. goveniana.*
30	~500 bp	multi-copy in *H. goveniana.*
46	~460 bp	prep band too low yield to sequence.
56	871 bp	clean seq. in *H. goveniana.* and *Juniperus.* No differences between *Hesperocyparis* and *Juniperus*! NADH dehydrogenase subunit 5, mitochondrion gene *A. thaliana*, EU999005
57	946 bp	1 SNP (J. *virg-scop*); 20 SNPs (J. *virg-H. gov.*) *A. thaliana* NM_124642, heat shock protein 90.1
69	~800 bp	multi-copy in *H. goveniana.*
87	648 bp	messy, multi-copy in *H. goveniana.*
94	~500 bp	multi-copy in *H. goveniana.*

LCN 6. Two bright bands were amplified (~1500, ~700 bp, Table 2). Both bands were prepped and sequenced.
Band 1:

~1500 bp band: 497 bp of clean sequence data (forward primer) was obtained from *H. goveniana*, but the reverse primer sequence was messy indicating multi-copy. A BLAST of the 497 bp

sequence to GenBank gave the highest match to Actin-11 from *A. thaliana* U27981.

Band 2:

> ~700 bp band: 340 bp of clean sequence obtained from the forward primer for *H. goveniana* with the balance of the sequence messy. This is probably a multi-copy gene.

LCN 56. 871 bp of clean sequence was obtained from *H. goveniana, J. ashei* and *J. virginiana*. No differences were found between *Hesperocyparis* and *Juniperus*! A blast of GenBank matched to NADH dehydrogenase subunit 5, a mitochondrion gene of *A. thaliana*, EU999005 with a 90% similarity. This is an easy section to amplify, but there was no variation between these two Cupressaceae genera. LCN 56 seems of limited use.

LCN 57:   946 bp of clean sequence data was obtained from *H. goveniana* and *J. virginiana*. These species differ by 20 SNPs. Amplification from *J. ashei, J. blancoi* and *J. scopulorum* gave messy sequences. New internal primers were designed based on the sequences from *H. goveniana* and *J. virginiana*. *Juniperus scopulorum* was successfully sequenced using these primer pairs:

STLvirg57F40   ATGCTTCCTTCTACAAGAGT
STLvirg57R957   TCATTATCCTCTCCATGTT
and
STLvirg57F40   ATGCTTCCTTCTACAAGAGT
STLvirg57R965   CTGAGCCTTCATTATCCT

Juniperus scopulorum and *J. virginiana* differed by only 1 bp SNP. A blast of the H. goveniana sequence in GenBank matched heat shock protein 90.1, *A. thaliana* NM_124642. This may be the most promising LCN gene region discovered in this study and may prove useful in phylogenetic studies in the Cupressaceae, but it does not appear promising for infra-specific analysis due to the low variation found between closely related *J. scopulorum* and *J. virginiana* (1 SNP).

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NEW COMBINATIONS IN *SENEGALIA* AND *VACHELLIA* (FABACEAE: MIMOSOIDEAE)

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ABSTRACT

Morphological and genetic differences separating the subgenera of *Acacia s.l.* and molecular evidence that the genus *Acacia s.l.* is polyphyletic necessitate transfer of species from *Acacia* subgenus *Aculeiferum* Vassal to *Senegalia* and from *Acacia* subgenus *Acacia* to *Vachellia*, resulting in six new combinations in the genus *Senegalia*: *S. angico* (Mart. in Colla) Seigler & Ebinger, **comb. nov.**; *S. brevispica* (Harms) Seigler & Ebinger, **comb. nov.**; *S. galpinii* (Burtt Davy) Seigler & Ebinger, **comb. nov.**; *S. incerta* (Hoehne) Seigler & Ebinger, *S. mellifera* (Vahl) Seigler & Ebinger, **comb. nov.**; *S. schweinfurthii* (Brenan & Excell) Seigler & Ebinger **comb. nov.**;and five in *Vachellia*: *V. erioloba* (E. Mey.) Seigler & Ebinger, **comb. nov.**; *V. grandicornuta* (Gerstner) Seigler & Ebinger, **comb. nov.**; *V. haematoxylon* (Willd.) Seigler & Ebinger, **comb. nov.**; *V. hockii* (De Wild) Seigler & Ebinger, **comb. nov.**; and *V. nebrownii* (Burtt Davy) Seigler & Ebinger, **comb. nov.** *Phytologia 92(1): 92-95 (April, 2010)*.

KEY WORDS: *Acacia sensu lato*, Fabaceae, Mimosoideae, *Senegalia, Vachellia*.

Morphological and genetic differences separating the subgenera of *Acacia s.l.* and molecular evidence that the genus *Acacia s.l.* is polyphyletic necessitate recognition of segregate genera and transfer of many *Acacia* species to these genera. A large number of

species of *Acacia* subgenus *Aculeiferum* must be referred to *Senegalia* Rafinesque and some of *Acacia* subgenus *Acacia* to *Vachellia* Wight & Arnott. For the following 11 taxa, this results in new combinations, in either the genus *Senegalia* or *Vachellia*:

1. **SENEGALIA ANGICO** (Mart. in Colla) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia angico* Mart. in Colla, Herb. pedem. 2: 268. 1834. – TYPE: BRAZIL. RIO GRANDE DO SUL (Martius 1843), Villa Nova (holotype: BM; isotype: P, probable isotype K). The situation is confusing because Bentham (1876) considered *Acacia angico* Martius to be a synonym of *Piptadenia rigida* Benth., J. Bot. (Hooker) 4: 338. 1842. [now recognized as *Parapiptadenia rigida* (Benth.) Brenan, Kew Bull. 17: 228. 1963.] based on Martius (1843).

2. **SENEGALIA BREVISPICA** (Harms) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia brevispica* Harms, Notizbl. Bot. Gart. Berl. 8: 370. 1923. – TYPE: TANZANIA: Lushoto Dist., Kitivo, *Holst 606* (holotype: B, probably destroyed; BM, drawing) (Ross 1979).

3. **SENEGALIA GALPINII** (Burtt Davy) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia galpinii* Burtt Davy, Bull. Misc. Inform. Kew. 326. 1922. – TYPE: SOUTH AFRICA. TRANSVAAL: banks of the Bad-zyn-loop River, Mosdene Estate, Naboomspruit, 19 Sep 1920, *Galpin 483M* (holotype: K; isotypes: BM, GRA, PRE) (Ross 1979).

4. **SENEGALIA INCERTA** (Hoehne) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia incerta* Hoehne, Com. lin. telegr., Bot., pt. 8: 22. (pl. 135). 1919. – TYPE: BRAZIL. MATTOGROSSO: em Copipo da ponte, Cuyaba, *F. C. Hoehne 2534* and *2535* (syntypes: possibly at R or RB). The numbers 2534 and 2535 are for the Commissão de Linhas Telegraphicas Estratégics de Matto Grosso ao Amazonas – CLTE and are not personal collection numbers (M. C. Mamede, personal communication).

5. **SENEGALIA MELLIFERA** (Vahl) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia mellifera* (Vahl) Benth. in Hook., Lond. J. Bot. 1: 507. 1842. – TYPE: ARABIA. Surdud and elsewhere, *Forsskal s.n.* (holotype: C) (Ross 1979).

6. **SENEGALIA SCHWEINFURTHII** (Burtt Davy) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia schweinfurthii* Brenan & Exell, Bol. Soc. Brot., ser. 2. 31: 128. t. 1, fig. E. 1957. – TYPE: SUDAN: Gubbiki. *Schweinfurth 2206* (holotype: BM; isotypes: HT, K, P, Z) (Ross 1979).

7. **VACHELLIA ERIOLOBA** (E. Mey.) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia erioloba* E. Mey., Comm. 1: 171. 1836. – TYPE: SOUTH AFRICA. TRANSVAAL: Namaqualand, Wolmaransstad Dist. Between Kommandodrif and Makwassie, *J. W. Morris 1042* [neotype: K; isotype: PRE] (Ross 1979).

8. **VACHELLIA GRANDICORNUTA** (Gerstner) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia grandicornuta* Gerstner, J. S. Afr. Bot. 4: 55. Fig. 1. 1938. – TYPE: SOUTH AFRICA. NATAL: 1) flowered at Emkunzana and Mkuzi Drift between Nongoma and Magudu, 6 Jan 1936, *Gerstner 2870* and 2) fruits found at same places and at lower Pongola, 13 May 1936, *Gerstner 2870* (syntypes: BOL) (Ross 1979).

9. **VACHELLIA HAEMATOXYLON** (Willd.) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia haematoxylon* Willd., Enum. Pl. 1056. 1809. – TYPE: SOUTH AFRICA. CAPE PROVINCE: *Lichtenstein s.n.* (holotype: B-Willd.) (Ross 1979).

10. **VACHELLIA HOCKII** (De Wild) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia hockii* De Wild, Reprium nov. Spec. Regni veg. 11: 502. 1913. – TYPE: ZAIRE. KATANGA: Luafu valley, *Hock s.n.* (holotype: BR) (Ross 1979).

11. **VACHELLIA NEBROWNII** (Burtt Davy) Seigler & Ebinger, **comb. nov.** Basionym: *Acacia nebrownii Burtt Davy,* Kew Bull. *1921: 50. 1921.* – TYPE: BOTSWANA: Kwebe Hills, *Mrs. E. J. Lugard 14* & 16 (syntypes: K) (Ross 1979).

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MISCELLANEOUS CHROMOSOME NUMBERS: TEXAS PLANT SPECIES

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ABSTRACT

Chromosome numbers are reported for 43 collections of flowering plant species in Texas; 26 species in 21 genera and 10 families are represented. First reports are presented for seven taxa: *Xanthisma gracile,* $2n = 3$ II; *Heliotropium molle,* $2n = $ 10-14 II; *Stanleya pinnata* var. *texana,* $2n = 14$ II; *Hermannia texana,* $2n = $ ca. 12 II (or 12 II + 1 I or fragment, or 13 II); *Aloysia gratissima,* $2n = $ ca. 54 (North America); *A. macrostachya,* $2n = 9$ II; *A. wrightii,* $2n = 27$ II. *Phytologia 92(1): 96-102 (April, 2010).*

KEY WORDS: Chromosome numbers, *Aloysia, Heliotropium, Hermannia, Stanleya, Xanthisma,* Texas species

Flower buds, fixed in modified Carnoy's Solution (4:3:1), and voucher specimens were obtained during general collecting activities in Texas during the years 2005-2009. Standard squash techniques (Turner

and Johnston, 1961) were used to obtain meiotic chromosome counts. Meiotic preparations for most of the species were evaluated by the first two authors, some of them by all three authors. Chromosome numbers obtained for most taxa listed below correspond with previous reports, as determined from the various chromosome number indexes and other literature. Voucher specimens are housed at SRSC and/or TEX-LL. Symbols and abbreviations: II, bivalents in meiotic configurations; I, univalent; III, trivalent; IV, quadrivalent; frag; fragment; *AMP*, A. M. Powell; *SAP*, Shirley A. Powell; *BLT*, B. L. Turner.

RESULTS AND DISCUSSION

ASTERACEAE
Erigeron tenuis Torr. & A. Gray
Bastrop Co., Bastrop State Park, *BLT* s.n., **2n = ca. 9 II** (possibly
     8 II = 2 or 3 round I's or frags.).
Grindelia nuda Alph. Wood var. **nuda**.
Hansford Co., ca. 0.8 km W of Gruver, *BLT 26-32*, **2n = 6 II**.
Haploesthes greggii A. Gray
Brewster Co., ca. 93 km S of Alpine along hwy 118, then 1 mi w along
     Agua Fria road, *BLT 25-144*, **2n = 18 II**.
Hymenopappus flavescens var. **canotomentosus** A. Gray
Jeff Davis Co., westernmost part, 13 km E of Culberson Co. line along
     hwy 90, *BLT 25-112*, **2n = 17 II**.
Pecos Co., 4.8 km S of Iraan, *BLT 25-83*, **2n = 17 II**.
Hymenoxys odorata DC.
Brewster Co., ca. 95 km S of Alpine, *BLT 7-1*, **2n = 11 II**.
Pecos Co., Sheffield cemetery, ca. 1.6 km W of town, *BLT 7-2*,
     2n = 11 II.
Sutton Co., E outskirts of Sonora, *BLT 7-16*, **2n = 12**.
     Sanderson and Strother (1973) reported counts of $2n = 11$ II, 12 II, and 14 II from Texas populations of *Hymenoxys odorata*. All of the $n = 12$ plants were reportedly from near Del Rio in Val Verde County; the $n = 12$ collection from Sutton County extends the range of the aneuploid populations.
Senecio riddellii Torr. & A. Gray
Brewster Co., 12 km S of Alpine along hwy 118, *S. F. Weyerts 16*,
     2n = 20 II.
Symphyotrichum expansum (Poepp. & Spreng.) G. L. Nesom

Brewster Co., ca. 14 km SE of Alpine, *AMP* and *SAP 6827*, **2n = 5 II**.
Thelesperma burridgeanum (Regel) S. F. Blake
Frio Co., red sandy soil along hwy, *BLT 7-8*, **2n = 10 II**.

As reviewed by Melchert (1963), but unpublished, the species was previously found to have 2n = 10 II.
Thelesperma longipes A. Gray
Schleicher Co., western part, where hwy 90 enters Crockett Co., *BLT 25-63*, **2n = 20 II**.
Thelesperma megapotamicum (Spreng.) Kuntze var.
    megapotamicum
Brewster Co., ca. 96 km S of Alpine along hwy 118, *BLT 25-141*,
    2n = 44 (18 II, 2 IV).

The count is consistent with previous reports (Melchert, 1963; Greer and Powell, 1999) for both *T. megapotamicum* var. *megapotamicum* and the "rayed form" var. *ambiguum* (A. Gray) Shinners which was also present at the site ca. 96 km S of Alpine.
Townsendia texensis Larsen
Potter Co., ca. 55 km NNE of Amarillo along hwy 136, *BLT 26-45*,
    2n = 9 II.
Xanthisma gracile (Nutt.) D. R. Morgan & R. L. Hartm.
Brewster Co., ca. 14 km SE of Alpine, *AMP* and *SAP 6829*, **2n = 2 II;
    2n = 3 II.**

Buds were obtained from a cluster of 3-4 morphologically identical plants with stems intermingled. One head of buds yielded a clear count of $2n = 3$ II, a first record of this chromosome number for the species in Texas. Examination of another head from the same collection resulted in a count of $2n = 2$ II, typical for the species. The circumstance of three-paired and two-paired cytological races in the same small population is similar to that described by Jackson (1965) for this species [as *Haplopappus gracilis* (Nutt.) A. Gray] in south-central Arizona.
Xanthisma spinulosum (Pursh) D. R. Morgan & R. L. Hartm. var.
    spinulosum.
Hansford Co., ca. 0.8 km W of Gruver, *BLT 26-33*, **2n = 4 II**.

BORAGINACEAE
Heliotropium molle (Torr.) I. M. Johnst.
Brewster Co., ca. 93 km S of Alpine along hwy 118, *BLT 25-151*,
    2n = 10-14 II.

Albeit equivocal, this is a first report for the species; the meiotic configurations, small, dim-staining chromosomes, were difficult to interpret; possibly the count should be 12 or 13 II because related taxa, such as *H. curassivicum*, possess 2n = 13 pairs.

BRASSICACEAE

Stanleya pinnata var. **texana** B. L. Turner

Brewster Co., ca. 93 km S of Alpine (at type locality), *BLT 25-145*,

    2n = 14 II.

This is a first report for var. *texana*; S. *pinnata* (Purph) Britton var. *pinnata* was previously reported as *2n* = 14 II (Rollins and Rudenberg, 1977).

Streptanthus carinatus C. Wright ex A. Gray

Jeff Davis Co., along FM 2017, ca. 17 km S of hwy 90, *BLT & M. Terry 25-1*, **2n = 14 II**.

COMMELINACEAE

Tradescantia pedicellata Celarier

Gillespie Co., 30 km N of Fredericksburg, *BLT 26-21*, **2n = 6 II +**

    1 centric frag.

The single, round fragment was consistently present in meiotic configurations, often near the end of one of the bivalents in a satellite-like position; the only other count for this seldom collected species has been that of Celarier (1965), who reported observations similar to our own.

FABACEAE

Dalea glaberrima S. Watson

El Paso Co., eastern part, where I-10 crosses into Hudspeth Co., *BLT 25-101*, **2n = 7 II**.

Dalea glaberrima is synonymous with *D. lanata* var. *terminalis* (M. E. Jones) Barneby, as treated by Turner (2006). Previous counts for the genus have all been on a base number of $x = 7$.

Hoffmannseggia glauca (Ortega) Eifert

Brewster Co., ca. 93 km S of Alpine, *BLT 25-148*, **2n = ca. 12 II**.

Meiotic configurations exhibited irregularities, including some possible multivalents, and two laggards at anaphase I.

KRAMERIACEAE
Krameria grayi Rose & Painter
Hudspeth Co., central part of Van Horn Mountains, *BLT & M. Terry, s. n.*, **2n = ca. 6 II**.

LAMIACEAE
Warnockia scutellarioides (Engelm. & A. Gray) M. W. Turner
Williamson Co., just NW of Round Rock, *Matt Turner 121*,
    2n = ca. 10-11 II.
    Turner (1996) previously reported *n* = 10 and *n* = ca. 10 for the species.

POLEMONIACEAE
Phlox drummondii var. **mcallisteri** Wherry
Gillespie Co., 30 km mi N of Fredericksburg, *BLT 26-26*, **2n = 7 II**.

STERCULIACEAE
Hermannia texana A. Gray
Uvalde Co., 30 km mi NE of Brackettville *BLT 26-6*, **2n = ca. 12 II (or 12 II = 1 I or frag.; or 13 II)**.
    This is a first report for the species; interpretation of meiotic configurations was equivocal because of heteromorphic bivalents, possible multivalents (i.e., 1-2 III's), and 1-2 I's, as suggested above.

VERBENACEAE
Aloysia gratissima (Gillies & Hook.) Tronc.
Brewster Co., Alpine, *BLT* s. n., **2n = ca. 48-54**.
Brewster Co., 8 km W of Alpine, *BLT* s. n., **2n = ca. 54**.
Brewster Co., Alpine, Sul Ross campus, *B. L. Turner* s. n., **2n = ca. 54**.
Crockett Co., ca. 5 km E of Sheffield, *BLT 26-28*, **2n = ca. 54**.
Gillespie Co., 30 km N of Fredericksburg, *BLT 26-19*, **2n = ca. 54**.
Kinney Co., ca. 5 km NE of Brackettville, *BLT 26-2*, **2n = ca. 70-72**.
Kinney Co., ca. 19 km NE of Brackettville, *BLT 26-3*, **2n = ca. 54**.
Llano Co., 16 km S of Llano along hwy 16, *BLT 26-23*, **2n = ca. 54**.
Mason Co., 12 km E of Mason, *BLT 26-24*, **2n = ca. 54**.
Presidio Co., Pinto Canyon, *M. Terry 0908-2006*, **2n = ca. 54**.
Travis Co., Austin, just E of Zilker Park, *BLT 26-14*, **2n = ca. 54**.
Travis Co., Austin, Univ. of Texas campus, near Turtle Ponds, *S. J. Siedo 729*, **2n = ca. 48-54**.

Uvalde Co., 16 km S of Camp Wood, *BLT 26-23*, **2n = ca. 54**.
Val Verde Co., 17.2 km E of Del Rio, *BLT 26-25*, **2n = ca. 50-54**.
Aloysia macrostachya (Torr.) Moldenke
Travis Co., Austin, Univ. of Texas campus, near Green House, *S. J. Siedo 840*, **2n = 9 II**.
Aloysia wrightii A. Heller ex Abrams
Val Verde Co., 17.2 km E of Del Rio, *BLT 26-26*, **2n = 27 II**.

As reviewed by Siedo (2006), where pertinent literature is cited, the base chromosome number of *Aloysia* Palau is $x = 9$, first suggested with the (1943) count of $2n = 36$ for the South American *A. citriodora* Palau. Subsequent published counts for the genus (year in parentheses), all South American, were: $2n = 18$ II (1946), *A. ligustrina* (Lag.) Small (cultivated, identified as this species, but instead probably *A. lycioides* as construed by Siedo); $2n =$ ca. 72 (1961), *A. scorodonioides* (Kunth) Moldenke; $2n = 18$ II (1972), *A. lycioides* Cham.; $2n = 54$ (1998), *A. gratissima*; $2n = 54$ (1998), *A. polystachya*; 2n=36 (1998).

The first documented counts for North American populations of *Aloysia* are listed above (present paper). *Aloysia wrightii* is apparently hexaploid, as inferred by the single count of $2n = 27$ II. *Aloysia gratissima* is mostly hexaploid, as suggested by the 13 counts, each approximately $2n = 54$; this is consistent with the one previous South American report for the amphitropical, *A. gratissima*. One North American count for *A. gratissima* from Kinney County was octoploid, $2n =$ ca. 70-72. Our report for *A. macrostachya*, $2n = 9$ II, is apparently the first to document the presumed base number for the genus. Meiosis in *A. gratissima* was always seemingly irregular, with bivalents, multivalents, univalents, laggards, and subsequent micronuclei; chromosomes were relatively small and dim-staining.

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FORMAL TRANSFER OF *EUPATORIUM HINTONIORUM* B. L. TURNER TO *KOANOPHYLLON* (ASTERACEAE: EUPATORIEAE)

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ABSTRACT

Eupatorium hintoniorum B.L. Turner is formally transferred to the genus *Koanophyllon* Arruda as **K. hintoniorum** (B.L. Turner) Velazco, comb. nov. *Phytologia 92(1): 103-104 (April, 2010).*

KEY WORDS: *Eupatorium*, *Koanophyllon hintoniorum*, Eupatorieae, Asteraceae, Nuevo Leon, Mexico.

During a search of information regarding endemic plant species in the state of Nuevo Leon (Mexico) and adjacent states, it came to the author's attention that no bibliographical reference showed the formal transfer of *Eupatorium hintoniorum* B.L. Turner into *Koanophyllon*, although the name *K. hintoniorum* has been used in several papers such as Villarreal y Estrada (2008), Turner (2009) and Velazco (2009). The name is also accepted within *Koanophyllon* by The National Commission of Biodiversity in México (CONABIO, 2008).

Eupatorium hintoniorum was described in the same publication as *E. galeanum* (Turner, 1992), and while the latter was formally transferred to *Koanophyllon* (Turner, 1997), *E. hintoniorum* was not. When searching the Tropicos database (www.tropicos.org) the query returns "*Koanophyllon hintoniorum* B. L. Turner" as an accepted name for *E. hintoniorum* but no bibliographic reference is shown. The International Plant Name Index site (www.ipni.org) gives no data when searching for *K. hintoniorum*, but only for *E. hintoniorum*.

In order to maintain uniformity among *Koanophyllon* species and give proper bibliographic reference, the following combination is proposed:

Koanophyllon hintoniorum (B. L. Turner) Velazco, **comb. nov.**
BASIONYM: *Eupatorium hintoniorum* B.L. Turner, Phytologia 72(5): 367-368. 1992.

ACKNOWLEDGEMENTS

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JUNIPERUS ZANONII, A NEW SPECIES FROM CERRO POTOSI, NUEVO LEON, MEXICO

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ABSTRACT

Analyses of nrDNA and trnC-trnD sequence data revealed that the shrubby alpine juniper from Cerro Potosi is not allied with *J. monticola* f. *compacta* from Ixtaccihuatl in the trans-volcanic belt of central Mexico, but forms a distinct clade with *J. saltillensis*. Research using Single Nucleotide Polymorphisms (SNPs) with samples of *J. monticola* f. *compacta* from the near the type locality confirmed that the Cerro Potosi alpine-subalpine juniper is not related to either *J. monticola* f. *monticola* nor *J. m.* f. *compacta*. A new species is proposed, ***Juniperus zanonii*** R.P. Adams, **sp. nov.** from Cerro Potosi and nearby alpine-subalpine mountains of NE Mexico. The alpine-subalpine *J. monticola* junipers of the trans-volcanic belt in central Mexico were shown to be in a different phylogenetic group than *J. zanonii*. *Phytologia 92(1): 105-117 (April, 2010).*

KEY WORDS: *Juniperus jaliscana, J. monticola, J. saltillensis, J. zanonii*, nrDNA, trn C-trnD, petN-psbM, SNPs, Cupressaceae

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Analyses of nrDNA and trnC-trnD sequences (Adams et al. 2008) of the serrate leaf margined junipers of the western hemisphere has revealed some un-expected phylogenetic information. Figure 1 shows a Bayesian tree based on nrDNA and trnC-trnD sequences for this group. Notice that the alpine shrubby juniper from Cerro Potosi (3490 m) is in a well support clade with *J. saltillensis*. However, *J.*

monticola (f. *monticola* from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*.
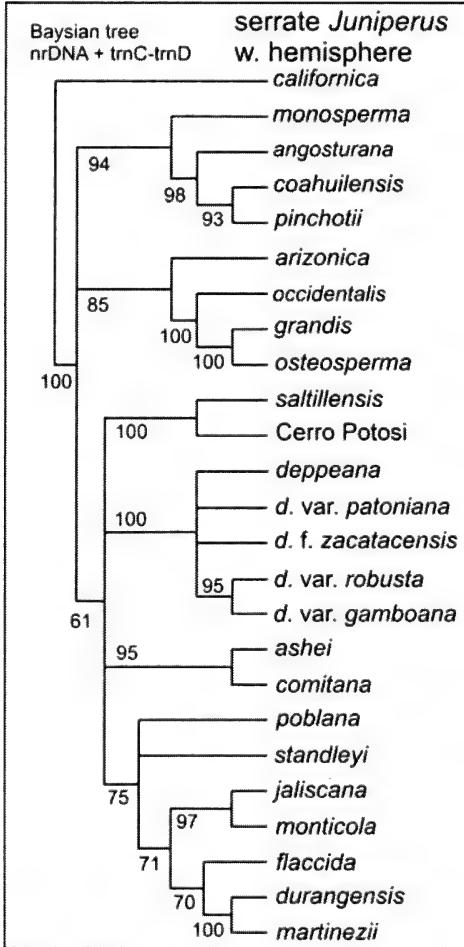
Figure 1. Bayesian tree for the serrate-leave *Juniperus* of the western hemisphere (modified from Adams, 2008).

Notice that the Cerro Potosi juniper is in a well support clade with *J. saltillensis*, whereas *J. monticola* (f. *monticola*) from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*. But, *J. m.* f. *compacta* from Popocatepetl (nor nearby Ixtaccihuatl) was not included in this analyses.

The alpine juniper on Cerro Potosi has been treated as *J. monticola* f. *compacta* by many authors (Adams, 2008; Farjon, 2005; Martinez, 1693; McDonald, 1990, 1993; Zanoni and Adams, 1975, 1976, 1979). So it is not surprising that Adams et al. (2007) treated the Cerro Potosi juniper as a disjunct population of *J. monticola* f.

compacta. As previously mentioned, the alpine-subalpine juniper shrub from Cerro Potosi, Nuevo Leon was found to be so distinct in its nrDNA and cp trnC-trnD that it was recognized at the specific level as *J. compacta* (Mart.) R. P. Adams (=*J. monticola* f. *compacta* Mart.) (Adams et al., 2007). However, because the holotype for *J. monticola* f. *compacta* is from Volcan Popocatepetl (*Martinez 7003*) and the habitats there differ from Cerro Potosi (where it grows as an understory plant beneath *Pinus culminicola* and *P. hartwegii* on limestone) and Popocatepetl (where it is found above timberline, on volcanic material, in full sunlight), it seemed of interest to compare these populations with putative *J. monticola* f. *compacta*. In addition, Zanoni and Adams (1976) examined the leaf oils of plants from Cerro Potosi and Ixtaccihuatl (their *J. m.* f. *compacta* population was only about 16 km from the Popocatepetl type locality). They reported that the Cerro Potosi plant oils were very different from *J. m.* f. *compacta*, *J. m.* f. *monticola* and *J. m.* f. *orizabensis* populations from the central Mexico volcanoes, but they did not follow up on these data.

The purpose of this study was to compare materials of *J. monticola* f. *compacta* from near the type locality with materials from the shrubby alpine-subalpine juniper from Cerro Potosi using sequencing of nrDNA and the petN-psbM cp DNA. *Juniperus jaliscana*, *J. saltillensis* and *J. virginiana* were included as outgroups.

MATERIALS AND METHODS

Specimens collected: *J. jaliscana*, *Adams 6846-6848*, 12/12/1991, 940 m, 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. monticola* f. *compacta*: *Alicia Mastretta, 1-4*, I1-I4, (= *Adams 11738-11740*), 19° 10' N, 98° 38' W, 4270 m, Pico Ixtaccihuatl, Mexico; putative *J. m. f. compacta*, *Adams 6898-6902*, 12/21/1991, 3490 m, Cerro Potosi, Nuevo Leon, Mexico; *J. monticola* f. *monticola*, *Adams 6874-6878*, 12/20/1991, 2750 m, El Chico National Park, Hidalgo, Mexico; *J. monticola* f. *orizabensis*: *S. Gonzalez 7243a,b, 7244a,b,* O1-O4 (=*Adams 11267-11270*), Pico de Orizaba, Vera Cruz, Mexico; *J. saltillensis*, *Adams 6886-6890*, 12/21/1991, 2090 m, on Mex. 60, 14 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. virginiana*, *Adams 10230-10232*, Knoxville, TN. Voucher specimens are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).   ITS (nrDNA), petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments   and   NJ   trees   were   made   using   MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/).   Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).   Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Phylogenetic analyses, based on combined nrDNA plus petN-psbM (cp DNA) sequences, revealed that the alpine-subalpine juniper shrubs from Cerro Potosi are in a clade with *J. saltillensis* and not with *J. monticola* f. *compacta* (Fig. 2).   Morphologically, it is difficult to distinguish the Cerro Potosi juniper shrubs from *J. m.* f. *monticola* at Popocatepetl (and Ixtaccihuatl) as both taxa have compact foliage, with reduced leaves at the high elevation sites. It is easy to understand why Adams et al. (2007) failed to include *J. m.* f. *compacta* from Popocatepetl (or Ixtaccihuatl) in their DNA analyses. This led them to assume that the Cerro Potosi alpine-subalpine juniper was in fact *J. m.* f. *compacta* and

led to the elevation of *J. m.* f. *compacta* to *J. compacta*. The results from the Bayesian tree (Fig. 2) clearly show that both *J. m.* f. *monticola* (Hidalgo, El Chico NP) and *J. m.* f. *compacta* (Ixtaccihuatl) are not phylogenetically closely related to the alpine-subalpine juniper of Cerro Potosi.
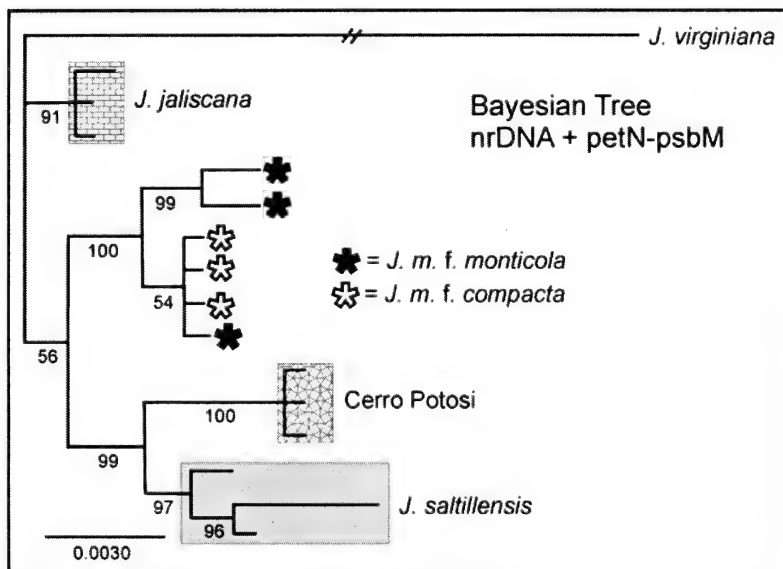
Figure 2. Bayesian tree based on combined sequences from nrDNA and petN-psbM (cp DNA). Numbers at the branch points are posterior probabilities.

Analyses of the nrDNA sequences revealed 20 mutations (nucleotide differences plus indels), of which 7 occurred only once. The remaining 13 mutations were treated at SNPs for analysis. A minimum spanning network (Figure 3, left) shows the Cerro Potosi junipers to be well resolved from both *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola* (El Chico Natl. Park, Hidalgo).

Analysis of the petN-psbM sequences revealed 17 mutations (nucleotide differences plus indels), of which 3 occurred only once. The remaining 14 mutations were treated at SNPs for analysis. A minimum

spanning network (Figure 3, right) revealed the Cerro Potosi junipers to be well resolved from both *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola* (El Chico Natl. Park, Hidalgo). The petN-psbM SNPs did not separate *J. monticola* f. *compacta* (Ixtaccihuatl) from *J. m.* f. *monticola* (Figure 3, right).
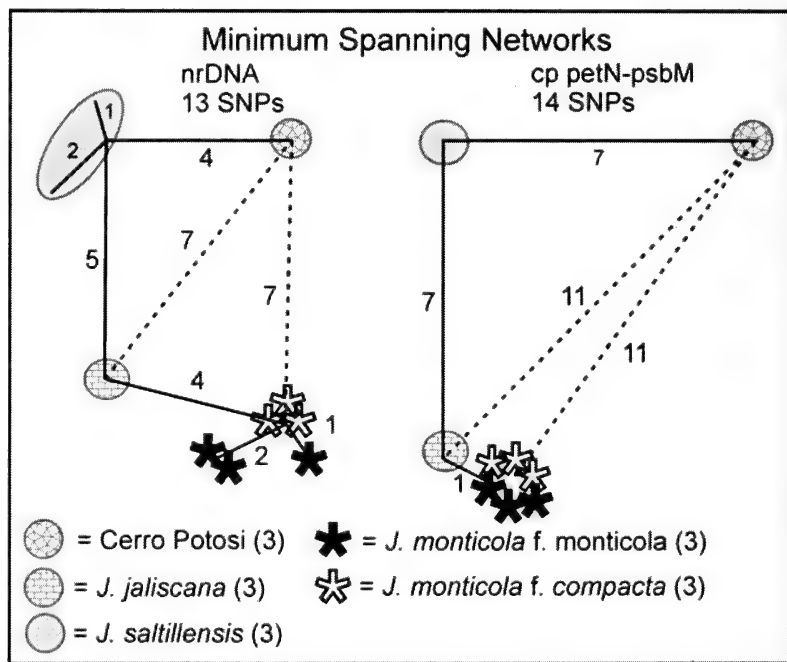
Figure 3. Minimum spanning networks based on nrDNA (left) and petN-psbM (right). The numbers next to the lines (links) are the number of SNPs. The dashed lines are the next shortest link from the Cerro Potosi junipers to other taxa. The numbers in parenthesis after the names are the number of samples analyzed per taxon.

Combining the 13 SNPs from nrDNA and 14 SNPs from cp petN-psbM sequences shows the additive nature of these two gene regions (Figure 4). The Cerro Potosi junipers are clearly very differentiated from *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola*; in short, the common application of *J. m.* f. *monticola* to the

alpine junipers of Cerro Potosi is not supported. The recognition (Adams et al., 2007) of the Cerro Potosi juniper as a part of *J. m.* f. *compacta* and the elevation of *J. m.* f. *compacta* to *J. compacta* (Mart.) R. P. Adams is not supported. The juniper populations at alpine-subalpine habitats on Cerro Potosi and adjacent areas are not closely related to *J. monticola* f. *compacta*, but represent a new species, more related to *J. saltillensis*.
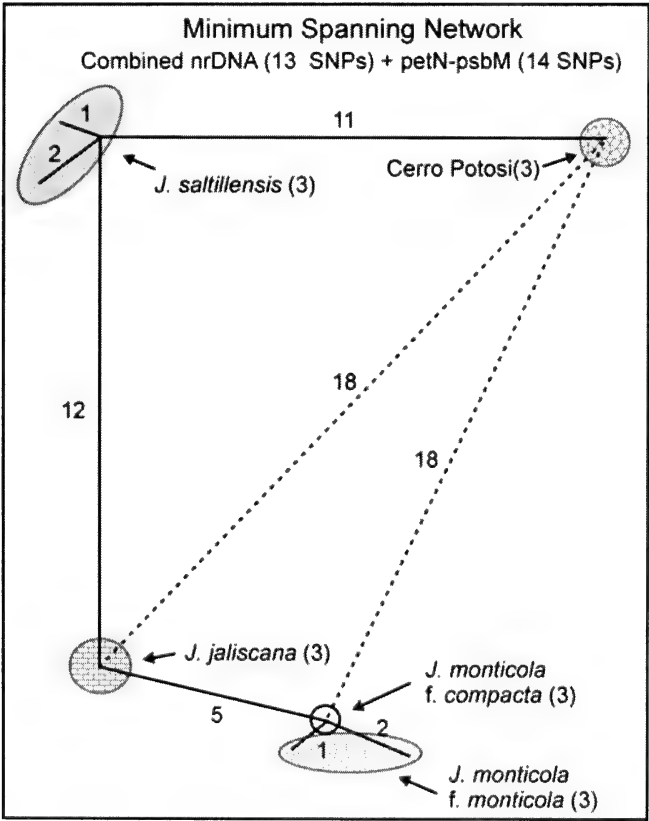
Figure 4. Minimum spanning network using 27 SNPs combined from nrDNA and petN-psbM. See figure 2 for details.

A new alpine-subalpine species in NE Mexico is recognized as:

Juniperus zanonii R.P. Adams, **sp. nov.** Type: Mexico, Nuevo Leon, Cerro Potosi, 3550 m, *J. A. McDonald 1820*, 26 Jul 1985 (HOLOTYPE: TEX).

J. monticolae forma *compactae* similis sed differt caulibus ramulorum (3-5 mm in diam.) cortice asperi foliis emortuis persistentibus, foliis squamatis saepe porcatis glandibus ubi manifestis complanatis vel elevatis ovalibus, et foliis flagelliformibus glandibus elevatis ovalibus vel elongatis.

This species is similar to *J. monticola* f. *compacta* but differs in that the branchlet stems (3 -5 mm diam.) have rough bark and persistent dead leaves, scale-leaves with flat or raised oval glands when visible, scale-leaves often with a ridge, and whip-leaves with raised, oval to elongate glands.

Table 1 shows some characteristic differences between *J. zanonii* and *J. monticola* f. *compacta.* Although the DNA shows *J. zanonii* to be more closely related to *J. saltillensis* than to *J. monticola*, the morphology of *J. zanonii* is nearly identical to *J. monticola.* Of course, it may be that the shorter, colder growing season in the alpine-subalpine areas leads to similar, compact foliage in both taxa.

The new species is named after Thomas A. Zanoni (1949-, presently at NYBG). Tom was my first Ph. D. student and spent much time in Mexico collecting *Juniperus*. Tom and I visited (again) the top of Cerro Potosi to collect junipers in 1991 and, fortunately, I put samples in silica gel for DNA analysis, as we did not do that in his work in the 1970s. Tom led some great field trips in Mexico and Guatemala.

McDonald (1990, 1993) examined and mapped the alpine-subalpine vegetation of NE Mexico. Although the extent of the areas is limited (Fig. 5), floristic affinities were shown (McDonald, 1990) to be strong among these areas and not similar to that of the trans-volcanic belt of central Mexico. McDonald (1993) used the Sorenson index of genetic similarity between alpine-subalpine vegetations and showed that the NE Mexico alpine-subalpine flora was more similar to that of the White

Mtns., NM, U. S. A., than to that of the trans-volcanic belt of central Mexico.

McDonald (1993) argues that the alpine zones extended downward about 1000 m during the Wisconsin glacial maximum, leading to much larger alpine-subalpine areas in NE Mexico. However, even if the alpine-subalpine zone descended 1000 m, the alpine-subalpine areas of NE Mexico were not continuous with either the Trans-Pecos, Texas Mtns, or the trans-volcanic belt of central Mexico (McDonald, 1993).

Table 1. Morphological differences between *J. zanonii* and *J. monticola* f. *compacta*.

	J. zanonii	*J. m.* f. *compacta*
Branchlets (3-5 mm diam.)	rough, with persistent dead leaves.	smooth
Scale leaves	often with ridge on beak	domed beak, no ridge
Scale leaf glands (when visible)	oval, flat or raised	elongated, sunken groove on leaf
Whip leaf glands	oval to elongated raised or flat	ill-defined elongated, sunken to flat
Habitat	limestone, under *Pinus culminicola* and *P. hartwegii* near timberline	volcanic lava and rocks above timberline

At present, *J. zanonii* is known from Sierra La Viga, Sierra La Marta, Cerro Potosi and Sierra Pena Nevada (Fig. 5) of the seven alpine-subalpine areas of McDonald (1990, 1993). It seems likely that *J. zanonii* occurs on the three other alpine-subalpine areas (S. Potrero de Abrego, S. Coahuilon, and S. Borrado, Fig. 5).
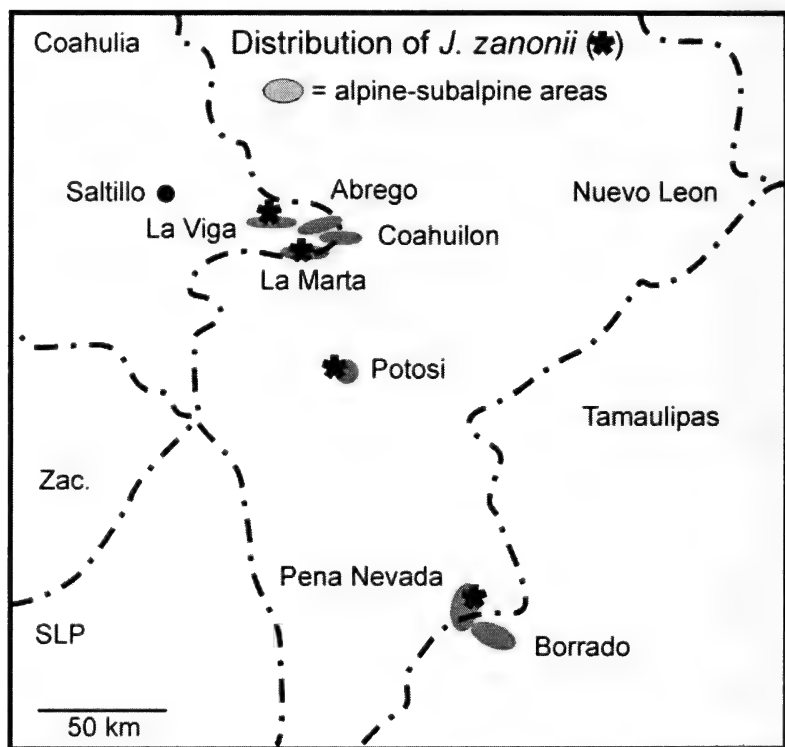
Figure 5.   Distribution of *J. zanonii* overlaid onto a map of alpine-subalpine areas in NE Mexico based on McDonald, 1990, 1993. Alpine areas: La Viga (Sierra La Viga); Abrego (Sierra Potrero de Abrego); Coahuilon) Sierra Coahuilon); La Marta (Sierra La Marta); Potosi (Cerro Potosi); Pena Nevada (Sierra Pena Nevada) and Borrado (Sierra Borrado).

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Appendix I. Specimens examined.

J. zanonii
Coahuila: Cerro de Viga, *Henrickson, et al. 16130b, 16143* (TEX); *Hinton et al. 20201* (TEX); Sierra La Marta *McDonald 2140* (TEX). **Nuevo Leon**: Cerro Potosi, *Adams 6898-6902* (BAYLU); *Gilbert 13* (TEX); *Marroquin 346* (TEX); *McDonald 1820* (TEX); *Mueller 2262* (TEX); *Schneider 963* (A, ARIZ, F, MICH, MO, NA); *Zanoni 2588, 2589, 2590, 2591, 2592* (BAYLU); *Zanoni 2589, 2594, 2597, 2598* (TEX). **Tamaulipas**: Cerro Pena Nevada summit. *Stanford, et al. 2571* (DS, RSA).

J. monticola **f.** *compacta (=J. compacta)*
Coahuila: Sierra Mojada, *M. E. Jones 191* (MO, POM, RSA, US). Location may be mis-labeled?
District Federal: La Cima station, Serjana de Ajusco, *Beaman 2807* (GH, MSC).
Jalisco: Nevada de Colima, *Brizuela* 27 Oct 1967 (ENCB).
Mexico: Tlaloc, *Beaman 2316* (GH, MSC); Ixtaccihuatl, Mexico, *Alicia Mastretta, 1-4,* L1-L4, (= *Adams 11906-11909*, BAYLU); Volcan Popocatepetl, *Martinez 7003* (MEXU), the recent eruption of Popocatepetl may have destroyed this population. *Koeppen. & Iltis 1030* (MICH, MSC, TEX, UC); Nevada de Toluca, *Zanoni 2199, 2208* (TEX), *Beaman 1721* (GH, MSC)
Tlaxacala: La Malinche, *Beaman 2248* (MICH).
Vera Cruz: Cofre de Perote, *Martinez 10525* (BH, F) and *10524b* (MO, UC); *S. Gonzalez 7240a, b, c* (BAYLU);

J. monticola **f.** *monticola*
District Federal: La Cima station, Serjana de Ajusco, *Koeppen. & Iltis* 12-14 Jul 1960 (TEX).

Guerrero: Cerro Teotepec, Mpio. Tlacotepec, *Rzedowski 18574* (MICH, TEX).
Hidalgo: Real del Monte, *Martinez 10523* (F, RSA); El Chico National Park, *Adams 6874-6878* (BAYLU).
Jalisco: Nevado de Colima, *Gregory & Eiten 300* (MICH, MO, SMU).
Michoacan: Pico Tancitaro, *Leavenworth & Hoogstral 1163A* (F, MO).
Morelos: km. 42 on Mexico - Cuernavaca road, *Reko & Halbinger 65* (A).
Mexico: Monte de las Cruces, *Martinez 10523A* (DS), *Martinez 10524* (UC).
San Luis Potosi: Puerto de Cedros, 3 km e of Mineral de Catorce, *Rzedowski 7270* (ENCB).

J. monticola f. *orizabensis*
Vera Cruz: Pico de Orizaba, *Beaman 1756* (GH, MSC, US); *S. Gonzalez 7243a,b, 7244a,b* (BAYLU); *Martinez 10526* (MO, RSA, UC); *Smith 488* (MO); *Balls 4394* (A, UC, US); *Balls 4619* (A, US); Sierra Nigra (sw of Pico de Orizaba), *Beaman 2519* (GH, MSC, UC).

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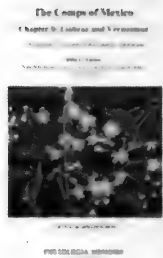
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VASCULAR FLORA OF THE ROCKY FLATS AREA, JEFFERSON COUNTY, COLORADO, USA

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ABSTRACT

The Rocky Flats Site (Site) is a U.S. Department of Energy (DOE) facility near Golden, Colorado that produced nuclear weapons components during the Cold War. Like many federal properties that have been off-limits to public access for decades, it has become a refugia for biodiversity as surrounding landscapes have been lost to agriculture and urbanization. A floristic study of the area was conducted on approximately 2,505 ha (6,189 ac) and includes the parcels currently managed and operated by DOE and the U.S. Fish and Wildlife Service (Rocky Flats National Wildlife Refuge). A flora of 630 species of vascular plants in 84 families and 340 genera was documented, including 12 species endemic to the southern Rocky Mountains and seven species considered rare or imperiled by the Colorado Natural Heritage Program. The flora of the Site is characterized by a predominantly Western North American floristic element, however, an Adventive floristic element contributes the greatest number of species. The vegetation is dominated by xeric tallgrass prairie and mixed grass prairie, with areas of wetland, shrubland, and riparian woodland. *Phytologia 92(2): 121-150 (August 2, 2010).*

KEY WORDS: Colorado, flora, phytogeography, refugia, Rocky Flats Site, Rocky Flats National Wildlife Refuge, U.S. Department of Energy, xeric tallgrass prairie

Government installations on otherwise undeveloped lands have received increased attention in recent years as refugia of biological diversity (Cohn, 1994; Gray and Rickard, 1989; Mann et al., 1996; Nickens, 1993). Many U.S. Department of Defense (DoD) and U.S. Department of Energy (DOE) lands contain protected habitat for

endangered, threatened, or sensitive plants, animals, and plant communities. Unlike national parks and forests, DoD and DOE lands have been off-limits, often serving as buffer areas separating facility operations from the general public for security reasons and protection from potential contaminant releases. As surrounding landscapes have been modified and changed by agriculture and urban development, many of these buffer areas have become large islands of relatively undisturbed biotic communities.

The DOE Rocky Flats Site (Site) between Golden and Boulder, Colorado, is one such area. Formerly known as the Rocky Flats Environmental Technology Site, the facility produced nuclear weapons components for nearly 40 years during the Cold War, from the early 1950s through 1989. From 1989 to 2005, the Site underwent environmental cleanup under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), more commonly known as Superfund. In 2001, prior to completion of cleanup activities, Congress enacted the Rocky Flats National Wildlife Refuge Act to set aside most of the Site as the Rocky Flats National Wildlife Refuge (RFNWR) once the Environmental Protection Agency certified that cleanup and closure activities were complete. Cleanup and closure of the Site concluded in October 2005. In July 2007, approximately 1597 ha (3947 ac) of the Site was transferred to the U.S. Fish and Wildlife Service (USFWS) to become the RFNWR. DOE retained the area where the former industrial complex was located, now known as the Central Operable Unit (COU), while most of the surrounding buffer area, known as the Peripheral Operable Unit (POU), was transferred to the USFWS.

For most of the last 50 years, the POU has remained largely undisturbed. During the 1970s and 1980s, a few ecological and environmental studies were conducted by researchers at the University of Colorado at Boulder and Colorado State University in Fort Collins, Colorado (Arthur and Alldredge, 1982; Clark et al., 1980; Weber et al., 1974; Little et al., 1980). Beginning in the early 1990s, ecological monitoring was conducted as part of the cleanup operations to ensure compliance with environmental laws and regulations, provide baseline ecological information, monitor resources, and provide technical support and data for ecological resource management. Over the years,

the vascular flora was inventoried to document the diversity of the plant life present at the Site.   Ecological monitoring continues today for natural resource management as revegetation and habitat restoration return the area to a more natural state.   This paper documents the vascular flora of the Rocky Flats area, encompassing both the COU and POU, and discusses the floristic and phytogeographic characteristics of the flora.

STUDY AREA

Located 25.7 km (16 mi) northwest of downtown Denver, the Site, approximately 2,505 ha [6,189 ac] in size, is located on the Colorado Piedmont approximately 3.2 km (2 mi) east of the mountain front between Golden and Boulder, Colorado. The Site is located at approximately 39° 53' N latitude and 105° 12' W longitude. Elevations range from 1,707 m (5,600 ft) on the eastern edge of the Site to 1,884 m (6,180 ft) along the western edge. The topography consists of gently east-sloping flat pediment (mesa) tops that have been dissected by intermittent and ephemeral streams, resulting in moderate to steep hillsides. The surface geologic unit of the mesa tops, the Rocky Flats Alluvium, is a glacial outwash fan originating from Coal Creek canyon to the west. The soils are classified as Nederland very cobbly sandy loams on the mesa tops interspersed with units of clay loams on hillslope and valley bottoms (SCS, 1980). According to records from the nearby National Renewable Energy Laboratory, the average annual precipitation is approximately 38 cm (15 in), most of which falls during April and May. The mean monthly temperature ranges from a low of approximately 1° C (34° F) in January to a high of approximately 22° C (71° F) during July. High winds, sometimes in excess of 145 km/hr (90 mph), frequently buffet the Site during the winter months.

METHODS

An early botanical inventory conducted by Weber et al. (1974) documented a flora of 327 vascular plants, 16 mosses, and 25 lichens at the Site.   This inventory, deposited at the University of Colorado herbarium (COLO) in Boulder provided an initial species list for the Site. During the 1990s, dominant plant communities at the Site were inventoried and mapped as part of the ongoing ecological monitoring

program. The unpublished vegetation map and associated information was used for the Site plant community information presented in this report (K-H, 1997). Additional plant collections (through summer 2009) have provided a comprehensive inventory of the Site flora. Voucher specimens have been collected of all species documented at the Site. Specimens were collected, pressed and dried, identified, verified at COLO, and mounted using accepted herbarium techniques. The taxonomy used for this study follows GPFA (1986), Weber (1976), Weber (1990), and Weber and Wittman (2001), in that order of determination. The complete set of voucher specimens for the Site is housed at COLO.

Floristic summaries were compiled using the complete species list and included total species richness, total number of plant families, total number of genera, total number species by growth form, plant families and genera with the greatest number of species.

Plant geographers have long recognized that species have specific range distributions and that plant species can be grouped or classified based on their geographic distributions. When the taxa from a flora of a particular area or region are grouped based on similarities in worldwide distribution the resulting floristic classification categories are called phytogeographical elements (Peinado et al., 2009). A geographical analysis of the Rocky Flats species provided a representation of the phytogeographic elements that contribute to the flora of the Site. The geographical analysis followed the methodology used in recent regional floristic accounts by Hogan (1993), Clark (1996), and Nelson and Harmon (1997). Eleven elements were defined: Western North America (WNA), Great Plains (GP), Eastern North America (ENA), Southern Rocky Mountains (SR), Southwestern North America (SNA), North America (NA), Western Hemisphere (WH), Oroboreal (O), Circumpolar (C), Cosmopolitan (COS), and Adventive (A). Western North American species are generally distributed west of the 100th Meridian. Great Plains species are distributed across the central plains of North America. Distributions of Eastern North America species are generally east of the 100th Meridian. The Southern Rocky Mountains species are found in the Rocky Mountains from southern Wyoming and into Colorado and New Mexico. The Southwestern North American (Chihuahuan) species are

evidence of a Madrean influence. North American species are those distributed throughout most of the continent. Western Hemisphere species have distributions in North, Central, and South America. Oroboreal species have distributions across southern Canada, the northern United States, and south along the Appalachian mountains, and western Cordillera. Circumpolar species are those found throughout the Holarctic in North America, Europe, and Asia. Cosmopolitan species have a worldwide distribution. Adventive species are defined as native and non-native species that tend to grow in weedy, disturbed areas. Where possible, species were assigned elements based on determinations made previously by Hogan (1993), Clark (1996), Nelson (1993), and Nelson and Harmon (1997). Various floras were used to determine distributions of species not previously assigned element status, (Fernald, 1950; Gleason and Cronquist, 1963; GPFA, 1986; Hitchcock, 1971; Rydberg, 1932; USDA, NRCS, 2009).

RESULTS AND DISCUSSION

Plant Communities

The dominant plant communities at the Site can be divided into eight types: xeric tallgrass prairie, mesic mixed grassland, needle and threadgrass prairie, Great Plains riparian woodland, willow and wild indigo shrublands, tall upland shrubland, wetlands, and reclaimed grasslands.

Xeric tallgrass prairie. The xeric tallgrass prairie is considered to be a relict plant community from the last glaciation. This rare and unique prairie occurs only in a narrow band on the Colorado Piedmont, east of the mountain front in Colorado. The xeric tallgrass prairie on the Site, combined with that on City of Boulder Open Space to the west, is believed to be the largest remaining tract of this plant community in North America (CNHP, 1994, 1995). It covers approximately 733 ha (1,811 ac) at the Site and contains a unique mixture of tallgrass and montane species. Many of the tallgrass species are not commonly found between the mountain front and the true tallgrass prairie of the eastern Great Plains several hundred miles east. Dominant species within this community at the Site include *Andropogon gerardii* Vitman, *Muhlenbergia montana* (Nutt.) Hitchc., *Aster porteri* Gray, and *Poa*

compressa L. Other common species include *Stipa comata* Trin. & Rupr., *Sporobolus heterolepis* (A. Gray) A. Gray, *Andropogon scoparius* Michx., *Carex heliophila* Mack., *Liatris punctata* Hook., *Chrysopsis villosa* Pursh., *Arenaria fendleri* A. Gray, *Sorghastrum nutans* (L.) Nash, *Bouteloua gracilis* (H. B. K.) Lag ex Griffiths, and *Bouteloua curtipendula* (Michx.) Torr. Two rarer species found on the xeric tallgrass prairie include *Carex oreocharis* Holm. and *Stipa spartea* Trinius.

Mesic mixed grassland. The mesic mixed grassland covers the greatest area at the Site (approximately 896 ha [2,213 ac]) and is most abundant on the hillsides. It is classified by the presence of *Agropyron smithii* Rydb., *B. gracilis*, *B. curtipendula* and *B. japonicus* Thunb. ex Murr. Other common species include *Poa pratensis* L., *Stipa viridula* Trin., *Psoralea tenuiflora* Pursh, *Ratibida columnifera* (Nutt.) Woot. & Standl., and *Alyssum minus* (L.) Rothmaler var. *micranthus* (C. A. Mey.) Dudley.

Needle and threadgrass prairie. The needle and threadgrass prairie covers approximately 76 ha [189 ac] and is typically found on the eastern edges of the pediments. Dominant species are *Stipa comata*, and two exotic species, *Linaria dalmatica* (L.) Mill. and *B. japonicus*. Other commonly encountered species include *Stipa neomexicana* (Thur.) Scribn., *Poa pratensis*, *P. compressa*, *Yucca glauca* Nutt., *C. heliophila*, *B. gracilis*, *B. curtipendula*, and *A. minus*.

Great Plains riparian woodland. The Great Plains riparian woodland is found along reaches of all the streams at the Site and is classified primarily by the presence of a *Populus deltoides* Marsh. ssp. *monilifera* (Ait.) Eckenw. canopy. It only accounts for approximately 11 ha (28 ac) due to the intermittent and ephemeral nature of the the streams at the Site. Other canopy species include *Populus angustifolia* James, *Salix amygdaloides* Anderss., and *Acer negundo* L. var. *interius* (Britt.) Sarg. The understory often contains *Salix exigua* Nutt. ssp. *exigua*, *Amorpha fruticosa* L., *Juncus balticus* Willd., *Cirsium arvense* (L.) Scop., *Bromus inermis* Leyss. ssp. *inermis*, *Carex nebrascensis* Dew., and a variety of other forbs and graminoids.

Willow and wild indigo shrublands. These shrublands are common along the streams in the valleys at the Site and often occur adjacent to the Great Plains riparian woodland. These shrublands cover approximately 17 ha (41 ac). They are classified by the presence of *S. exigua* and *A. fruticosa*, both of which can occur singly as dominants or together as co-dominants. Other common species include *J. balticus*, *C. arvense*, *C. nebrascensis*, *Typha latifolia* L., *Geranium caespitosum* James ssp. *caespitosum*, and *Agrostis stolonifera* L.

Tall upland shrubland. The tall upland shrubland is classified by the presence of *Prunus virginiana* L. var. *melanocarpa* (A. Nels.) Sarg., *Crataegus erythropoda* Ashe, and *Prunus americana* Marsh. These shrublands are considered unique to the Site and region, and they are found associated with the upper edges of hillside seep wetlands (CNHP, 1994). These often narrow, linear communities are common in the Rock Creek drainage on predominantly north-facing slopes at the Site. Although representing less than 1 percent of the total area of the Site (14 ha, 34 ac), more than 50 percent of the Site's flora is found in association with this community. Several plant species are found only associated with this community at the Site, including *Hydrophyllum fendleri* (Gray) Heller, *Osmorhiza chilensis* H. & A., *Osmorhiza longistylis* (Torr.) DC var. *longistylis*, *Smilax herbacea* L. var. *lasioneura* (Small) Rydb., *Viola rydbergii* Greene, *Viola scopulorum* (Gray) Greene, *Cystopteris fragilis* (L.) Bernh., *Physocarpus monogynus* (Torr.) Coult., *Lupinus argenteus* Pursh ssp. *ingratus* (Greene) Harmon, *Lupinus argenteus* Pursh var. *argenteus*, and *Agropyron griffithsii* Scribn. & Smith.

Wetlands. Wetlands at the Site are found along the streams and the hillside seep-fed wetlands on the north-facing slopes in the Rock Creek and Woman Creek drainages. The seeps emerge on the hillsides and form the wetlands at the juncture where the Rocky Flats Alluvium meets the bedrock. The wetlands cover approximately 165 ha (407 ac) at the Site. These wetlands are dominated by *J. balticus*, *C. arvense*, *Carex lanuginosa* Michx., and *T. latifolia*. Other common species include *Geum macrophyllum* Willd., *Barbarea vulgaris* R. Br., *C. nebrascensis*, *Asclepias incarnata* L., *Mentha arvensis* L., *Juncus longistylis* Torr., *Spartina pectinata* Link, and *Nasturtium officinale* R. Br.

Reclaimed grasslands. Two types of reclaimed grasslands occur at the Site. They are distinguished by the seed mixes that were used for reclamation. Areas seeded prior to the 1990s were seeded predominantly with exotic graminoid species and are dominated by *B. inermis* and *Agropyron intermedium* (Host) Beauv., with a few locations of *Agropyron cristatum* (L.) Gaertn. Little native vegetation is present at many of these reclaimed grasslands, even after more than a quarter-century (Nelson, 1999). These reclaimed areas account for approximately 261 ha (645 ac). Newly seeded reclamation grasslands have been seeded with native species, common to the native prairie communities at the Site. These areas are dominated by *Agropyron smithii*, *A. caninum* (L.) Beauv. ssp. *majus* (Vasey) C.L. Hitchc., *B. gracilis*, *B. curtipendula*, *Buchloe dactyloides* (Nutt.) Engelm., *A. gerardii*, *S. nutans*, and *Panicum virgatum* L. These areas cover approximately 263 ha (650 ac).

Floristics

The vascular flora of the Site consists of 630 species distributed across 84 families and 340 genera, including 5 pteridophytes, 5 gymnosperms, and 620 angiosperms. The checklist of the vascular flora of the Site is provided below. Seventy-six percent of the flora is composed of native species. The growth habits of the flora include 145 graminoids, 421 forbs, 32 shrubs, 24 trees, 6 cacti, and 2 vines. The plant families that contribute the greatest number of species to the flora are the Asteraceae (108 species), Poaceae (101 species), Fabaceae (34 species), Cyperaceae (31 species), Rosaceae (28 species), Brassicaceae (28 species), and Scrophulariaceae (24 species). Twelve species endemic to Colorado and the southern Rocky Mountains occur at the Site (Maley, 1994; Weber, 1976; Weber and Wittman, 1992): *A. porteri*, *Cryptantha virgata* (Porter) Payson, *Erigeron vetensis* Rydb., *Harbouria trachypleura* (Gray) C. & R., *Lithospermum multiflorum* Torr., *Penstemon secundiflorus* Benth., *Penstemon virens* Penn., *Penstemon virgatus* Gray ssp. *asa-grayi* Crosswhite, *Physaria vitulifera* Rydb., *Potentilla fissa* Nutt., *Scutellaria brittonii* Porter, and *Senecio fendleri* Gray.

No federally listed threatened or endangered plant species have been found at the Site; however, seven species of special concern

as listed by the CNHP are present (CNHP, 2009): *C. oreocharis*, *Aristida basiramea* Engelm. ex Vasey var. *basiramea*, *S. herbacea*, *Triodanis leptocarpa* (Nutt.) Nieuw., *Equisetum variegatum* Schleich., *Asclepias stenophylla* A. Gray, and *Amorpha nana* Nutt.

Phytogeography

The Site flora is predominantly Western North American in distribution (145 sp./23.0%; Table 1); however, the North American (89 sp./14.1%) and Great Plains (82 sp./13.8%) elements also contribute substantially to the flora. The high representation of Western North American and Great Plains elements is not unexpected given the ecotonal position of the Site at the juncture of the western prairie edge and foothills of the Rocky Mountains. One of the more interesting components of the Site flora is the Eastern North American element (31 sp./4.9%). It has been suggested that this element, comprising eastern woodland/prairie species, spread westward and contacted the Rocky Mountain region during the last glacial period (Weber 1965, 1976). These relict species remained in isolated refugia after the last ice age, in the cooler ravines and montane environments found in a narrow band along the eastern mountain front of Colorado. Many of the tallgrass prairie species found at the Site belong to this group, including *A. gerardii*, *A. scoparius*, *S. heterolepis*, *S. spartea*, and *S. nutans*. Other species at the Site that belong to this element include *Lobelia siphilitica* L. var. *ludoviciana* A. DC., *Ceanothus herbaceus* Raf. var. *pubescens* (T. & G.), *Agrimonia striata* Michx., *Helianthus rigidus* (Cass.) Desf. ssp. *subrhomboideus* (Rydb.) Heiser, and *Lysimachia ciliata* L. (Hogan, 1993; Maley, 1994; Weber, 1976).

One of the most striking discoveries of this study was that the Adventive element—both native and non-native species that prefer disturbed, weedy areas—contributed the highest number of species to the flora of the Site (166 sp./26.4%). The relatively high contribution of the Adventive element has also been documented in other recent regional floristic surveys. In the city of Boulder Mountain Parks, Hogan (1993) found that the Adventive element contributed 21.1% of the flora. Maley (1994) found that 17.3% of the flora of the Black Forest area between Denver and Colorado Springs came from the the Adventive element, while Clark found only a 12.9% contribution in the

Mesa de Maya region of southeastern Colorado. At higher elevations, the contribution of the Adventive element has been less than at lower elevations. In the Gore Range of central Colorado, Hogan (1992) reported the Adventive element contributed only 4% of the flora. In general, this would suggest that the percentage of Adventive element species in a local flora seems to be lower where there has been less impact from humans.

The significance of the Adventive element may be somewhat exaggerated, however, because species lists note only presence or absence, and do not provide any measure of abundance. Most of the Adventive element species at the Site do not occur in great numbers, and many are based on single collections. Several of these species have been found in recent years in the reclaimed grassland areas, most likely as seed mix contaminants. In most cases it is rare that they persist beyond one or two years. Some of the Adventive element species, however, present one of the greatest threats to, and challenges for management of the plant communities at the Site—noxious weeds. The noxious weeds that are currently problematic include *Centaurea diffusa* Lam., *C. arvense*, *L. dalmatica* (L.) Mill., *Carduus nutans* L. ssp. *macrolepis* (Peterm.) Kazmi, *Euphorbia uralensis* Fisch. ex Link, *Onopordum acanthium* L., and *Verbascum thapsus* L.

CONCLUSIONS

The floristic richness at the Site is very high and diverse considering the relatively small size of the area. Much of this can be attributed to the ecotonal position of the Site at the juncture of the Great Plains and the foothills of the Rocky Mountains. The high representation of Western North American and Great Plains species supports this. The xeric tallgrass prairie, Great Plains riparian woodland, tall upland shrubland, and wetland communities and their respective floras found at the Site represent a rich heritage of what much of the Colorado Piedmont east of the mountain front in Colorado must have looked like in the past. This study documents the known vascular flora present at the Site and provides a baseline for future comparisons. As development and urbanization continue to transform the native landscape, areas like Rocky Flats continue to serve as refugia for plants and animals that were once much more common.

ACKNOWLEDGEMENTS

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Table 1. Phytogeographical element summary of the flora of the Rocky Flats area.

Phtygeographical Elements	Number of Species	Percent of Flora
Western North America (WNA)	145	23.0
North America (NA)	89	14.1
Great Plains (GP)	87	13.8
Eastern North America (ENA)	31	4.9
Southern Rocky Mountains (SR)	24	3.8
Circumpolar (C)	24	3.8
Southwestern North America (SNA)	23	3.7
Oroboreal (O)	17	2.7
Cosmopolitan (COS)	16	2.5
Western Hemisphere (WH)	8	1.3
Adventive (A)	166	26.4
Totals	630	100

CHECKLIST OF THE VASCULAR FLORA OF THE ROCKY FLATS AREA

Nomenclature follows GPFA (1986), Weber (1976), Weber (1990), Weber and Wittmann (2001), in that order of determination. The letters behind each species denote the phytogeographic element classification and follow the acronyms as defined in the text.

ACERACEAE
Acer glabrum Torr., WNA; *Acer negundo* L. var. *interius* (Britt.) Sarg., NA; *Acer platanoides* L., A

AGAVACEAE
Yucca glauca Nutt., GP

ALISMATACEAE
Alisma triviale Pursh, NA; *Sagittaria latifolia* Willd., NA

AMARANTHACEAE
Amaranthus albus L., NA; *Amaranthus graecizans* L., WNA; *Amaranthus retroflexus* L., A

ANACARDIACEAE
Rhus aromatica Ait. var. *trilobata* (Nutt.) A. Gray, WNA; *Toxicodendron rydbergii* (Small) Greene, NA

APIACEAE
Berula erecta (Huds.) Cov. var. *incisum*, NA; *Cicuta maculata* L. var. *angustifolia* Hook., NA; *Conium maculatum* L., A; *Daucus carota* L., A; *Harbouria trachypleura* (Gray) C. & R., SR; H*eracleum sphondylium* L. ssp. *montanum* (Schleich.) Briq., NA; *Ligusticum porteri* C. & R., SR; *Lomatium orientale* Coult. & Rose, GP; *Musineon divaricatum* (Pursh.) Nutt. var. *hookeri* T. & G., GP; *Osmorhiza chilensis* H. & A., O; *Osmorhiza longistylis* (Torr.) DC var. *longistylis*, O

APOCYNACEAE
Apocynum androsaemifolium L., NA; *Apocynum cannabinum* L., A

ASCLEPIADACEAE
Asclepias incarnata L., ENA; *Asclepias pumila* (Gray) Vail, GP;
Asclepias speciosa Torr., WNA; *Asclepias stenophylla* A. Gray, GP;
Asclepias viridiflora Raf., ENA

ASTERACEAE
Achillea millefolium L. ssp. *lanulosa* (Nutt.) Piper, WNA; *Agoseris glauca* (Pursh.) Dietr., WNA; *Ambrosia artemisiifolia* L., A; *Ambrosia psilostachya* DC., GP; *Ambrosia trifida* L., A; *Antennaria microphylla* Rydb., WNA; *Antennaria parvifolia* Nutt., WNA; *Anthemis cotula* L., A; *Arctium minus* Bernh., A; *Arnica fulgens* Pursh., WNA; *Artemisia campestris* L. ssp. *caudata* (Michx.) Hall & Clem., NA; *Artemisia dracunculus* L., WNA; *Artemisia frigida* Willd., NA; *Artemisia ludoviciana* Nutt. var. *ludoviciana*, NA; *Aster campestris* Nutt., WNA; *Aster falcatus* Lindl., WNA; *Aster fendleri* A. Gray, GP; *Aster hesperius* A. Gray var. *hesperius*, SNA; *Aster laevis* L. var. *geyeri* A. Gray, NA; *Aster porteri* Gray, SR; *Bidens cernua* L., A; *Bidens frondosa* L., A; *Carduus nutans* L. ssp. *macrolepis* (Peterm.) Kazmi, A; *Centaurea cyanus* L., A; *Centaurea diffusa* Lam., A; *Centaurea repens* L., A; *Centaurea solstitialis* L., A; *Chrysanthemum leucanthemum* L., A; *Chrysopsis fulcrata* Greene, WNA; *Chrysopsis villosa* Pursh., WNA; *Chrysothamnus nauseosus* (Pall.) Britt. ssp. *graveolens* (Nutt.) Piper, WNA; *Chrysothamnus nauseosus* (Pall.) Britt. ssp. *nauseosus*, WNA; *Cichorium intybus* L., A; *Cirsium arvense* (L.) Scop., A; *Cirsium flodmannii* (Rydb.) Arthur, GP; *Cirsium ochrocentrum* A. Gray, GP; *Cirsium undulatum* (Nutt.) Spreng., GP; *Cirsium vulgare* (Savi) Ten., A; *Conyza canadensis* (L.) Cronq., A; *Coreopsis tinctoria* Nutt., A; *Cosmos bipinnatus* Cav., A; *Crepis occidentalis* Nutt., WNA; *Crepis runcinata* (James) T. & G., WNA; *Dimorphotheca aurantiaca* D.C., A; *Dyssodia papposa* (Vent) Hitchc., NA; *Erigeron canus* A. Gray, GP; *Erigeron compositus* Pursh var. *dicoideus* A. Gray, WNA; *Erigeron divergens* T. & G., WNA; *Erigeron flagellaris* A. Gray, GP; *Erigeron pumilus* Nutt., WNA; *Erigeron speciosa* (Lindl.) DC. var. *macranthus* (Nutt.) Cronq., WNA; *Erigeron strigosus* Muhl. ex Willd., A; *Erigeron vetensis* Rydb., SR; *Gaillardia aristata* Pursh., WNA; *Gnaphalium chilense* Spreng., A; *Grindelia squarrosa* (Pursh.) Dun., GP; *Gutierrezia sarothrae* (Pursh.) Britt. & Rusby, WNA; *Haplopappus spinulosus* (Pursh) DC., GP; *Helianthus annuus* L., NA; *Helianthus ciliaris* DC., SNA; *Helianthus maximilianii* Schrad., GP;

Helianthus nuttallii T. & G., WNA; *Helianthus petiolaris* Nutt., GP;
Helianthus pumilus Nutt., SR; *Helianthus rigidus* (Cass.) Desf. ssp.
subrhomboideus (Rydb.) Heiser, GP; *Heliomeris multiflora* Nuttall,
WNA; *Hymenopappus filifolius* Hook. var. *cinereus* (Rydb.) I. M.
Johnst., GP; *Iva axillaris* Pursh., WNA; *Iva xanthifolia* Nutt., GP;
Kuhnia chlorolepis Woot. & Standl., SNA; *Kuhnia eupatorioides* L.,
ENA; *Lactuca oblongifolia* Nutt., WNA; *Lactuca serriola* L., A;
Leucelene ericoides (Torr.) Greene, WNA; *Liatris punctata* Hook., GP;
Lygodesmia juncea (Pursh.) Hook., GP; *Machaeranthera bigelovii*
(Gray) Greene, SNA; *Machaeranthera canescens* (Pursh) A. Gray,
WNA; *Madia glomerata* Hook., O; *Microseris cuspidata* (Pursh.) Sch.
Bip., GP; *Onopordum acanthium* L., A; *Picradeniopsis oppositifolia*
(Nutt.) Rydb., GP; *Ratibida columnifera* (Nutt.) Woot. & Standl., GP;
Rudbeckia ampla Nelson, ENA; *Scorzonera laciniata* L., A; *Senecio
fendleri* Gray, SR; *Senecio integerrimus* Nutt., WNA; *Senecio
plattensis* Nutt., GP; *Senecio spartioides* T. & G., WNA; *Senecio
tridenticulatus* Rydb., WNA; *Solidago canadensis* L., NA; *Solidago
gigantea* Ait., NA; *Solidago missouriensis* Nutt., WNA; *Solidago
mollis* Bart., GP; *Solidago nana* Nutt., WNA; *Solidago rigida* L., ENA;
Sonchus arvensis L. ssp. *uliginosus* (Bieb.) Nyman, A; *Sonchus asper*
(L.) Hill, A; *Stephanomeria pauciflora* (Torr.) A. Nels., GP;
Taraxacum laevigatum (Willd.) DC., A; *Taraxacum officinale* Weber,
A; *Thelesperma megapotanicum* (Spreng.) O. Ktze., WH; *Townsendia
grandiflora* (Nutt.), GP; *Townsendia hookeri* Beaman, GP; *Tragopogon
dubius* Scop., A; *Tragopogon porrifolius* L., A; *Verbesina encelioides*
(Cau) Benth. & Hook. ssp. *exauriculata* (Robins & Greenm.) Coleman,
A; *Xanthium strumarium* L., A

BERBERIDACEAE
Berberis repens Lindl., WNA

BETULACEAE
Alnus incana (L.) Moench ssp. *tenuifolia* (Nuttall) Breitung, WNA;
Betula occidentalis Hook., WNA

BORAGINACEAE
Asperugo procumbens L., A; *Cryptantha virgata* (Porter) Payson, SR;
Cynoglossum officinale L., A; *Hackelia floribunda* (Lehm.) I. M.
Johnst., WNA; *Lappula redowskii* (Hornem.) Greene, COS;

Lithospermum incisum Lehm., O; *Lithospermum multiflorum* Torr., SR; *Mertensia lanceolata* (Pursh.) A. DC., WNA; *Onosmodium molle* Michx. var. *occidentale* (Mack.) Johnst., GP; *Plagiobothrys scouleri* (H. & A.) I. M. Johnst., WNA

BRASSICACEAE
Alyssum alyssoides (L.) L., A; *Alyssum minus* (L.) Rothmaler var. *micranthus* (C. A. Mey.) Dudley, A; *Arabis fendleri* (S. Wats.) Greene var. *fendleri*, SNA; *Arabis glabra* (L.) Bernh., A; *Arabis hirsuta* (L.) Scop. var. *pycnocarpa* (Hopkins) Rollins, NA; *Barbarea vulgaris* R. Br., A; *Camelina microcarpa* Andrz. ex DC., A; *Capsella bursa-pastoris* (L.) Medic., A; *Cardaria chalepensis* (L.) Hand-Mazz, A; *Cardaria draba* (L.) Desv., A; *Chorispora tenella* (Pall.) DC., A; *Conringia orientalis* (L.) Dum., A; *Descurainia pinnata* (Walt.) Britt., ENA; *Descurainia richardsonii* (Sweet) Schultz, O; *Descurainia sophia* (L.) Webb ex Prantl., A; *Draba nemorosa* L., A; *Draba reptans* (Lam.) Fern., NA; *Erysimum capitatum* (Nutt.) DC., WNA; *Erysimum repandum* L., A; *Hesperis matronalis* L., A; *Lepidium campestre* (L.) R. Br., A; *Lepidium densiflorum* Schrad., A; *Lesquerella montana* (A. Gray) Wats., GP; *Nasturtium officinale* R. Br., COS; *Physaria vitulifera* Rydb., SR; *Rorippa palustris* (L.) Bess. ssp. *hispida* (Desv.) Jonsell, WNA; *Sisymbrium altissimum* L., A; *Thlaspi arvense* L., A

CACTACEAE
Coryphantha missouriensis (Sweet) Britt. & Rose, GP; *Echinocereus viridiflorus* Engelm., GP; *Opuntia fragilis* (Nutt.) Haw., WNA; *Opuntia macrorhiza* Engelm., GP; *Opuntia polyacantha* Haw., GP; *Pediocactus simpsonii* (Engelm.) Britt. & Rose, WNA

CALLITRICHACEAE
Callitriche verna L., C

CAMPANULACEAE
Campanula rotundifolia L., C; *Lobelia siphilitica* L. var. *ludoviciana* A. DC., NA; *Triodanis leptocarpa* (Nutt.) Nieuw., GP; *Triodanis perfoliata* (L.) Nieuw., A

CANNABACEAE
Humulus lupulus L. var. *lupuloides* E. Small, SNA

CAPPARACEAE
Cleome serrulata Pursh., WNA; *Polansia dodecandra* (L.) DC. ssp. *trachysperma* (T. & G.) Iltis, WNA

CAPRIFOLIACEAE
Symphoricarpos occidentalis Hook., WNA; *Symphoricarpos oreophilus* Gray, WNA; *Viburnum opulus* L. var. *americanum* Ait, O

CARYOPHYLLACEAE
Arenaria fendleri A. Gray, SNA; *Cerastium arvense* L., C; *Cerastium brachypodum* (Engelm. ex A. Gray) Robins., NA; *Cerastium vulgatum* L., A; *Conosilene conica* (L.) Fourreau ssp. *conoidea* (L.) Love & Kjellqvist, A; *Gypsophila elegans* Bieb., A; *Paronychia jamesii* T. & G., GP; *Saponaria officinalis* L., A; *Silene antirrhina* L., A; *Silene drummondii* Hook., WNA; *Silene pratensis* (Raf.) Godr. & Gren, A; *Spergularia media* (L.) Presl., A; *Spergularia rubra* (L.) K. Presl., NA; *Stellaria longifolia* Muhl. ex Willd., C; *Vaccaria pyramidata* Medic., A

CERATOPHYLLACEAE
Ceratophyllum demersum L., C

CHENOPODIACEAE
Atriplex canescens (Pursh.) Nutt., WNA; *Chenopodium album* L., A; *Chenopodium atrovirens* Nutt., WNA; *Chenopodium berlandieri* Moq., NA; *Chenopodium botrys* L., A; *Chenopodium dessicatum* A. Nels., WNA; *Chenopodium fremontii* S. Wats., WNA; *Chenopodium glaucum* L., GP; *Chenopodium leptophyllum* Nutt. ex Moq., WNA; *Chenopodium overi* Aellen, WNA; *Kochia scoparia* (L.) Schrad., A; *Salsola iberica* Senn. & Pau., A

CLUSIACEAE
Hypericum majus (A. Gray) Britt., NA; *Hypericum perforatum* L., A

COMMELINACEAE
Tradescantia occidentalis (Britt.) Smyth, GP

CONVOLVULACEAE
Calystegia macounii (Greene) Brummitt, C; *Calystegia sepium* (L.) R. Br. ssp. *angulata* Brummitt, NA; *Convolvulus arvensis* L., A; *Evolvulus nuttallianus* R. & S., GP

CRASSULACEAE
Sedum lanceolatum Torr., WNA

CUPRESSACEAE
Juniperus communis L., C; *Juniperus scopulorum* Sarg., WNA

CUSCUTACEAE
Cuscuta approximata Bab., A

CYPERACEAE
Carex athrostachya Olney, WNA; *Carex aurea* Nutt., O; *Carex bebbii* (Bailey) Fern, O; *Carex brevior* (Dew.) Mack. ex Lunell., O; *Carex douglasii* F. Boott., WNA; *Carex eleocharis* Bailey, C; *Carex emoryi* Dew., ENA; *Carex filifolia* Nutt., WNA; *Carex heliophila* Mack., ENA; *Carex hystericina* Muhl. ex Willd., NA; *Carex interior* Bailey, NA; *Carex lanuginosa* Michx., NA; *Carex nebrascensis* Dew., WNA; *Carex oreocharis* Holm., SR; *Carex praegracilis* W. Boott., NA; *Carex rostrata* Stokes ex Willd., C; *Carex scoparia* Schkuhr. ex Willd., NA; *Carex simulata* Mack., WNA; *Carex stipata* Muhl., NA; *Carex vulpinoidea* Michx., NA; *Cyperus acuminatus* Torr. & Hook., NA; *Eleocharis acicularis* (L.) R. & S., C; *Eleocharis compressa* Sulliv., ENA; *Eleocharis macrostachya* Britt., COS; *Eleocharis obtusa* (Willd.) J.A. Schult., NA; *Eleocharis parvula* Link ex Boff. & Fingerbr. var. *anachaeta* (Torr.) Svens., WH; *Scirpus acutus* Muhl., NA; *Scirpus maritimus* L. var. *paludosus* (A. Nels.) Kukenth., COS; *Scirpus pallidus* (Britt.) Fern, NA; *Scirpus pungens* Vahl, NA; *Scirpus validus* Vahl, NA

ELAEAGNACEAE
Elaeagnus angustifolia L., A

EQUISETACEAE
Equisetum arvense L., COS; *Equisetum laevigatum* A. Br., NA; *Equisetum variegatum* Schleich., NA

EUPHORBIACEAE

Euphorbia dentata Michx., NA; *Euphorbia fendleri* T. & G., WNA; *Euphorbia marginata* Pursh., GP; *Euphorbia robusta* (Engelm.) Small, GP; *Euphorbia serpyllifolia* Pers., A; *Euphorbia spathulata* Lam., WNA; *Euphorbia uralensis* Fisch. ex Link, A; *Tragia ramosa* Nutt., WNA

FABACEAE

Amorpha fruticosa L., NA; *Amorpha nana* Nutt., GP; *Astragalus adsurgens* Pall. var. *robustior* Hook., WNA; *Astragalus agrestis* Dougl. ex G. Don, WNA; *Astragalus bisulcatus* (Hook.) A. Gray, GP; *Astragalus canadensis* L., NA; *Astragalus crassicarpus* Nutt., GP; *Astragalus drummondii* Dougl. ex Hook., NA; *Astragalus flexuosus* (Hook.) G. Don, GP; *Astragalus lotiflorus* Hook., GP; *Astragalus parryi* Gray, SR; *Astragalus shortianus* Nutt. ex T.&G., SR; *Astragalus spathulatus* Sheld., GP; *Astragalus tridactylicus* Gray, GP; *Coronilla varia* L., A; *Dalea candida* Michx. ex Willd. var. *oligophylla* (Torr.) Shinners., GP; *Dalea purpurea* Vent, GP; *Glycyrrhiza lepidota* Pursh., WNA; *Lathyrus eucosmus* Butters and St. John, SNA; *Lotus corniculatus* L., A; *Lupinus argenteus* Pursh ssp. *ingratus* (Greene) Harmon, SR; *Lupinus argenteus* Pursh var. *argenteus*, WNA; *Medicago lupulina* L., A; *Medicago sativa* L. ssp. *sativa*, A; *Melilotus alba* Medic., A; *Melilotus officinalis* (L.) Pall., A; *Oxytropis lambertii* Pursh., GP; *Psoralea tenuiflora* Pursh., GP; *Robinia pseudoacacia* L., ENA; *Thermopsis rhombifolia* var. *divaricarpa* (Nels.) Isely, SR; *Trifolium hybridum* L., A; *Trifolium pratense* L., A; *Trifolium repens* L., A; *Vicia americana* Muhl. ex Willd., NA

FUMARIACEAE

Fumaria vaillantii Lois, A

GENTIANACEAE

Gentiana affinis Griseb., WNA; *Swertia radiata* (Kell.) O. Ktze., WNA

GERANIACEAE

Erodium cicutarium (L.) L'Her., A; *Geranium caespitosum* James ssp. *caespitosum*, SNA

GROSSULARIACEAE
Ribes aureum Pursh, WNA; *Ribes cereum* Dougl., WNA; *Ribes inerme* Rydb., WNA

HALORAGACEAE
Myriophyllum exalbescens Fern., NA

HYDROPHYLLACEAE
Hydrophyllum fendleri (Gray) Heller, WNA; *Phacelia heterophylla* Pursh., WNA

IRIDACEAE
Iris missouriensis Nutt., WNA; *Sisyrinchium montanum* Greene, O

JUNCACEAE
Juncus articulatus L., C; *Juncus balticus* Willd., WNA; *Juncus bufonius* L., COS; *Juncus dudleyi* Wieg., NA; *Juncus ensifolius* Wikst. var. *montanus* (Englm.) C. L. Hitchc., WNA; *Juncus interior* Wieg., GP; *Juncus longistylis* Torr., WNA; *Juncus nodosus* L., GP; *Juncus torreyi* Cov., NA; *Juncus tracyi* Rydb., WNA

JUNCAGINACEAE
Triglochin maritima L., NA

LAMIACEAE
Dracocephalum parviflorum Nutt., NA; *Hedeoma hispidum* Pursh., ENA; *Lycopus americanus* Muhl. ex Barton, NA; *Lycopus asper* Greene, GP; *Marrubium vulgare* L., A; *Mentha arvensis* L., COS; *Monarda fistulosa* L. var. *menthifolia* (Grah.) Fern., GP; *Monarda pectinata* Nutt., SNA; *Nepeta cataria* L., A; *Prunella vulgaris* L., COS; *Salvia reflexa* Hornem., GP; *Scutellaria brittonii* Porter, SR; *Stachys palustris* L. ssp. *pilosa* (Nutt.) Epling, O

LEMNACEAE
Lemna minor L., COS

LILIACEAE
Allium cernuum Roth, NA; *Allium geyeri* S. Wats., WNA; *Allium textile* A. Nels. & Macbr., GP; *Asparagus officinalis* L., A; *Calochortus*

gunnisonii S. Wats., WNA; *Leucocrinum montanum* Nutt., WNA; *Smilacina stellata* (L.) Desf., NA; *Zigadenus venenosus* Wats. var. *gramineus* (Rydb.) Walsh ex Peck, WNA

LINACEAE
Linum perenne L. var. *lewisii* (Pursh.) Eat. & Wright, WNA; *Linum pratense* (Nort.) Small, WNA; *Linum puberulum* (Englem.) Heller, SNA

LYTHRACEAE
Ammannia robusta Herr & Regel., NA; *Lythrum alatum* Pursh., ENA

MALVACEAE
Malva neglecta Wallr., A; *Sidalcea candida* Gray, WNA; *Sidalcea neomexicana* Gray, WNA; *Sphaeralcea coccinea* (Pursh.) Rydb., GP; *Sphaeralcea parvifolia* A. Nelson, A

NYCTAGINACEAE
Mirabilis hirsuta (Pursh.) MacM., GP; *Mirabilis linearis* (Pursh.) Heimerl, GP; *Mirabilis nyctaginea* (Michx.) MacM., GP

OLEACEAE
Fraxinus pennsylvanica Marsh, A

ONAGRACEAE
Calylophus serrulatus (Nutt.) Raven, GP; *Epilobium ciliatum* Raf. ssp. *glandulosum* (Lehm.) Hock & Raven, NA; *Epilobium paniculatum* Nutt., WNA; *Gaura coccinea* Pursh., WNA; *Gaura parviflora* Dougl., WNA; *Oenothera albicaulis* Pursh, GP; *Oenothera flava* (A. Nels.) Garrett, WNA; *Oenothera howardii* (A. Nels.) W. L. Wagner, GP; *Oenothera villosa* Thunb. ssp. *strigosa* (Rydb.) Dietrich & Raven, WNA

ORCHIDACEAE
Habenaria hyperborea (L.) R. Br., O

OROBANCHACEAE
Orobanche fasciculata Nutt., WNA

OXALIDACEAE
Oxalis dillenii Jacq., COS

PAPAVERACEAE
Argemone polyanthemos (Fedde) G. Ownbey, GP; *Eschscholzia californica* Chamisso, A; *Papaver rhoeas* L., A

PINACEAE
Picea pungens Engelm., SR; *Pinus ponderosa* Laws, WNA; *Pseudotsuga menziesii* (Mirb.) Franco, WNA

PLANTAGINACE
Plantago lanceolata L., A; *Plantago major* L., A; *Plantago patagonica* Jacq., A

POACEAE
Aegilops cylindrica Host, A; *Agropyron caninum* (L.) Beauv. ssp. *majus* (Vasey) C. L. Hitchc., NA; *Agropyron cristatum* (L.) Gaertn., A; *Agropyron dasystachyum* (Hook.) Scribn., GP; *Agropyron desertorum* (Fisch.) Schult., A; *Agropyron elongatum* (Host) Beauv., A; *Agropyron griffithsii* Scribn. & Smith, WNA; *Agropyron intermedium* (Host) Beauv., A; *Agropyron repens* (L.) Beauv., A; *Agropyron smithii* Rydb., NA; *Agropyron spicatum* (Pursh) Scribn. and Sm., WNA; *Agrostis scabra* Willd., NA; *Agrostis stolonifera* L., A; *Alopecurus geniculatus* L., C; *Andropogon gerardii* Vitman, ENA; *Andropogon saccharoides* Sw. var. *torreyanus* (Steud.) Hack., A; *Andropogon scoparius* Michx., ENA; *Apera interrupta* (L.) Beauvois, A; *Aristida basiramea* Engelm. ex Vasey var. *basiramea*, GP; *Aristida purpurea* Nutt. var. *longiseta* (Steud.) Vasey, WNA; *Aristida purpurea* Nutt. var. *robusta* (Merrill) A. Holmgren & N. Holmgr, WNA; *Avena fatua* var. *sativa* (L.) Hausskn., A; *Bouteloua curtipendula* (Michx.) Torr., NA; *Bouteloua gracilis* (H. B. K.) Lag ex Griffiths, WNA; *Bouteloua hirsuta* Lag, GP; *Bromus briziformis* F. & M., A; *Bromus inermis* Leyss. ssp. *inermis*, A; *Bromus japonicus* Thunb. ex Murr., A; *Bromus tectorum* L., A; *Buchloe dactyloides* (Nutt.) Engelm., GP; *Calamagrostis stricta* (Timm.) Koel, C; *Cenchrus longispinus* (Hack.) Fern, WH; *Ceratochloa marginata* (Nees ex Stued.) Jackson, WNA; *Chloris virgata* Sw., GP; *Cynodon dactylon* (L.) Pers., A; *Dactylis glomerata* L., A; *Danthonia spicata* (L.) Beauv. ex R. & S., NA; *Dichanthelium*

linearifolium (Scribn.) Gould, ENA; *Dichanthelium oligosanthes* (Schultz) Gould var. *scribnerianum* (Nash) G, NA; *Digitaria sanguinalis* (L.) Scop., A; *Distichlis spicata* (L.) Greene var. *stricta* (Torr.) Beetle, WH; *Echinochloa crus-galli* (L.) Beauv., A; *Elymus canadensis* L., NA; *Elymus junceus* Fisch., A; *Eragrostis cilianensis* (All.) E. Mosher, A; *Eragrostis curvula* (Schrad.) Nees, A; *Eragrostis minor* Host, A; *Eragrostis pilosa* (L.) Beauv., A; *Eragrostis trichodes* (Nutt.) Wood, GP; *Festuca octoflora* Walt., NA; *Festuca ovina* L. var. *rydbergii* St. Yves, C; *Festuca pratensis* Huds., A; *Glyceria grandis* S. Wats. ex A. Gray, C; *Glyceria striata* (Lam.) Hitchc., NA; *Hordeum brachyantherum* Nevski, O; *Hordeum jubatum* L., COS; *Hordeum pusillum* Nutt., NA; *Koeleria pyramidata* (Lam.) Beauv., NA; *Leersia oryzoides* (L.) Sw., A; *Leptochloa fasicularis* (Lam.) A. Gray, WH; *Lolium perenne* L. var. *aristatum* Willd., A; *Lolium perenne* L. var. *perenne*, A; *Lycurus phleoides* H.B.K., SNA; *Muhlenbergia asperifolia* (Nees. & Mey.) Parodi, WNA; *Muhlenbergia filiformis* (Thurb.) Rydb., GP; *Muhlenbergia montana* (Nutt.) Hitchc., WNA; *Muhlenbergia racemosa* (Michx.) B. S. P., GP; *Muhlenbergia wrightii* Vasey, SNA; *Oryzopsis hymenoides* (R. & S.) Ricker, WNA; *Panicum capillare* L., A; *Panicum dichotomiflorum* Michx., NA; *Panicum virgatum* L., ENA; *Phalaris arundinacea* L., C; *Phleum pratense* L., A; *Phragmites australis* (Cav.) Trin. ex Steud., COS; *Poa bulbosa* L., A; *Poa canbyi* (Scribn.) Piper, O; *Poa compressa* L., A; *Poa fendleriana* (Steud.) Vasey, WNA; *Poa juncifolia* Scribn., WNA; *Poa palustris* L., C; *Poa pratensis* L., A; *Polypogon monspeliensis* (L.) Desf., A; *Schedonnardus paniculatus* (Nutt.) Trel., WH; *Secale cereale* L., A; *Setaria viridis* (L.) Beauv., A; *Sitanion hystrix* (Nutt.) Sm. var. *brevifolium* (Sm.) Hitchc., WNA; *Sorghastrum nutans* (L.) Nash, ENA; *Spartina pectinata* Link, NA; *Sphenopholis obtusata* (Michx.) Scribn., NA; *Sporobolus asper* (Michx.) Kunth, ENA; *Sporobolus cryptandrus* (Torr.) A. Gray, NA; *Sporobolus heterolepis* (A. Gray) A. Gray, ENA; *Sporobolus neglectus* Nash, ENA; *Stipa comata* Trin. & Rupr., WNA; *Stipa neomexicana* (Thur.) Scribn., SNA; *Stipa robusta* (Vasey) Scribn., SR; *Stipa spartea* Trinius, ENA; *Stipa viridula* Trin., GP; *Triticum aestivum* L., A; *X Agrohordeum macounii* (Vasey) Lepage, A

POLEMONIACEAE
Collomia linearis Nutt., WNA; *Gilia opthalmoides* Brand. ssp. *clokeyi* (Mason) A. & V. Grant, WNA; *Ipomopsis spicata* (Nutt.) V. Grant ssp.

spicata, WNA; *Microsteris gracilis* (Hook.) Greene, WNA; *Navarretia minima* Nutt., WNA

POLYGONACEAE
Eriogonum alatum Torr., WNA; *Eriogonum effusum* Nutt., GP;
Eriogonum jamesii Benth., SNA; *Eriogonum umbellatum* Torr., WNA;
Polygonum arenastrum Jord. ex Bor., A; *Polygonum convolvulus* L., A;
Polygonum douglasii Greene, WNA; *Polygonum hydropiper* L., A;
Polygonum lapathifolium L., A; *Polygonum pensylvanicum* L., A;
Polygonum persicaria L., A; *Polygonum ramosissimum* Michx., A;
Polygonum sawatchense Small, WNA; *Rumex acetosella* L., A; *Rumex crispus* L., A; *Rumex maritimus* L., WH; *Rumex obtusifolius* L., A;
Rumex salicifolius Weinm. ssp. *triangulivalvis* Danser, NA

POLYPODIACEAE
Cystopteris fragilis (L.) Bernh., COS

PORTULACACEAE
Claytonia rosea Rydb., WNA; *Portulaca oleracea* L., A; *Talinum parviflorum* Nutt., GP

POTAMOGETONACEAE
Potamogeton foliosus Raf., NA; *Potamogeton natans* L., C

PRIMULACEAE
Androsace occidentalis Pursh., WNA; *Dodecatheon pulchellum* (Raf.)
Merrill, WNA; *Lysimachia ciliata* L., NA

RANUNCULACEAE
Anemone cylindrica A. Gray, O; *Anemone patens* L., C; *Clematis hirsutissima* Pursh, WNA; *Clematis ligusticifolia* Nutt., WNA;
Delphinium nuttalianum Pritz. ex Walpers, WNA; *Delphinium virescens* Nutt. ssp. *penardii* (Huth) Ewan, GP; *Myosurus minimus* L.,
COS; *Ranunculus macounii* Britt., NA; *Ranunculus scleratus* L., A;
Ranunculus trichophyllus Chaix, C; *Thalictrum dasycarpum* Fisch. &
Ave-Lall, NA

RHAMNACEAE
Ceanothus fendleri A. Gray, SNA; *Ceanothus herbaceus* Raf. var. *pubescens* (T. & G.), ENA

ROSACEAE
Agrimonia striata Michx., ENA; *Amelanchier alnifolia* Nutt., WNA; *Crataegus erythropoda* Ashe, SR; *Crataegus succulenta* Link var. *occidentalis* (Britton) E. J. Palm., GP; *Geum aleppicum* Jacq., NA; *Geum macrophyllum* Willd., NA; *Physocarpus monogynus* (Torr.) Coult., SR; *Physocarpus opulifolius* (L.) Raf., ENA; *Potentilla arguta* Pursh, NA; *Potentilla fissa* Nutt., SR; *Potentilla gracilis* Dougl. ex Hook. var. *glabrata* (Lehm.) C. L. Hitchc., WNA; *Potentilla hippiana* Lehm., WNA; *Potentilla norvegica* L., C; *Potentilla paradoxa* Nutt., NA; *Potentilla pensylvanica* L., WNA; *Potentilla pulcherrima x hippiana*, WNA; *Potentilla rivalis* Nutt., WNA; *Prunus americana* Marsh., ENA; *Prunus pumila* L. var. *besseyi* (Bailey) Gl., GP; *Prunus virginiana* L. var. *melanocarpa* (A. Nels.) Sarg., NA; *Pyrus malus* L., A; *Rosa acicularis* Lindl., C; *Rosa arkansana* Porter, ENA; *Rosa woodsii* Lindl., WNA; *Rubus deliciosus* Torr., SNA; *Rubus idaeus* L. ssp. *sachalinensis* (Levl.) Focke var. *sachalinensis*, C; *Sanguisorba minor* Scop., A; *Sorbus scopulina* Greene, WNA

RUBIACEAE
Galium aparine L., A; *Galium septentrionale* Roemer & Schultes, C

SALICACEAE
Populus alba L., A; *Populus angustifolia* James, WNA; *Populus deltoides* Marsh. ssp. *monilifera* (Ait.) Eckenw., ENA; *Populus x acuminata* Rydb., GP; *Salix amygdaloides* Anderss., NA; *Salix exigua* Nutt. ssp. *exigua*, NA; *Salix exigua* Nutt. ssp. *interior* (Rowlee) Cronq., NA; *Salix fragilis* L., A; *Salix irrorata* Andersson, SNA; *Salix lutea* Nutt., WNA

SANTALACEAE
Comandra umbellata (L.) Nutt., WNA

SAXIFRAGACEAE
Heuchera parvifolia Nutt. ex T.& G., WNA; *Saxifraga rhomboidea* Greene, WNA

SCROPHULARIACEAE
Castilleja integra A. Gray, SNA; *Castilleja sessiliflora* Pursh., GP;
Collinsia parviflora Doug. ex Lindl., WNA; *Gratiola neglecta* Torr.,
NA; *Limosella aquatica* L., O; *Linaria canadensis* (L.) Dum. var.
texana (Scheele) Penn., NA; *Linaria dalmatica* (L.) Mill., A; *Linaria
vulgaris* Hill, A; *Mimulus floribundus* Dougl. ex Lindl., WNA;
Mimulus glabratus H. B. K. var. *fremontii* (Benth.) A. L. Grant, WH;
Penstemon albidus Nutt., GP; *Penstemon eatonii* A. Gray var. *eatonii*,
A; *Penstemon palmeri* A. Gray, A; *Penstemon secundiflorus* Benth.,
SR; *Penstemon strictus* Bentham in De Candolle, SR; *Penstemon virens*
Penn., SR; *Penstemon virgatus* Gray ssp. *asa-grayi* Crosswhite, SR;
Scrophularia lanceolata Pursh., O; *Verbascum blattaria* L., A;
Verbascum thapsus L., A; *Veronica americana* (Raf.) Schwein. ex
Benth., NA; *Veronica anagallis-aquatica* L., A; *Veronica catenata*
Penn., A; *Veronica peregrina* L. var. *xalapensis* (H. B. K.) St. John &
Warren, A

SELAGINELLACEAE
Selaginella densa Rydb., WNA

SMILACACEAE
Smilax herbacea L. var. *lasioneura* (Small) Rydb.., ENA

SOLANACEAE
Physalis heterophylla Nees, ENA; *Physalis pumila* Nutt. ssp. *hispida*
(Waterfall) Hinton, GP; *Physalis virginiana* P. Mill., ENA; *Quincula
lobata* (Torr.) Raf., SNA; *Solanum rostratum* Dun., A; *Solanum
triflorum* Nutt., SNA

TAMARICACEAE
Tamarix ramosissima Ledeb., A

TYPHACEAE
Typha angustifolia L., COS; *Typha latifolia* L., COS

ULMACEAE
Ulmus pumila L., A

URTICACEAE
Parietaria pensylvanica Muhl. ex Willd., NA; *Urtica dioica* L. ssp. *gracilis* (Ait.) Seland., NA

VERBENACEAE
Lippia cuneifolia (Torr.) Steud., WNA; *Verbena bipinnatifida* Nutt., SNA; *Verbena bracteata* Lag. & Rodr., A; *Verbena hastata* L., NA

VIOLACEAE
Hybanthus verticillatus (Ort.) Baill., GP; *Viola nuttallii* Pursh., WNA; *Viola rydbergii* Greene, WNA; *Viola scopulorum* (Gray) Greene, SNA; *Viola sororia* Willd., GP

VITACEAE
Vitis riparia Michx., ENA

ZYGOPHYLLACEAE
Tribulus terrestris L., A;

HELIOTROPIUM PROCUMBENS (BORAGINACEAE): NATIVE OR ADVENTIVE IN THE USA?

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ABSTRACT

Heliotropium procumbens is a widespread weedy species of North America, South America and elsewhere. It occurs in the Gulf Coastal Region (Texas, Mississippi and Louisiana) of the USA and the question is posed: is it a native or an adventive in that area? After comparing the time and location of the various Texas specimens assembled over the past 150 years or so, it is concluded that the species is native to the area concerned. *Phytologia 92(2): 151-155 (August 2, 2010).*

KEY WORDS: *Heliotropium procumbens*, Texas, Louisiana, Mexico, adventive.

Heliotropium procumbens Mill., an annual or short-lived perennial herb 10-70 cm high with white flowers arranged in terminal scorpioid, ebracteate, spikes is reported to be a "weedy plant" (Al-Shehbaz 1991) that "usually grows on damp alluvial soil in flood plains, pinelands, mud flats, waste land, fields, and coastal hammocks."

The type of the taxon is from South America (Colombia). It occurs throughout most of that continent and northwards into Central America and Mexico (Fig. 1), extending into the USA along the Gulf Coastal Regions (Fig. 2). Whether it is native to Texas and Louisiana is debatable, hence the title of the present paper.

Relatively early collections of the taxon are known from both Louisiana and Texas but are absent for the rest of the Gulf Coast, although Ward and Fantz (1977) report an introduced Florida (Calhoun Co.) collection, first made in 1976, where it is clearly adventive. The present paper is concerned with its distribution and/or adventive status in the state of Texas.

To this end, we have surveyed the literature and examined all of the collections on file at LL-TEX and recorded the earliest collections from the state, these listed in Table 1. As will be noted, the earliest known collection of *H. procumbens* was made in 1843 in eastern Texas (Austin Co.) by Lindheimer; the next earliest collection, also by Lindheimer, was made in central Texas (Comal Co.) in 1850. The distribution of the first 10 collections known to be from Texas is shown in Fig. 3. Thereafter, numerous collections were made over a large region of the state, as shown in Fig. 2.

Table 1, Early collections of *H. procumbens* from Texas.
(Arranged chronologically; data mostly from Johnston, 1967)

1. **Austin Co.,** *Lindheimer*, 1843
2. **Comal Co.,** *Lindheimer*, 1850
3. **Val Verde Co.,** *Schott,* 1851-52
4. **Harris Co.,** *Fisher*, 1872
5. **Hidalgo Co.,** *Neally,* 1889
6. **Uvalde Co.,** *Plank,* 1891
7. **Hays Co.,** *Stanfield*, 1896
8. **Cameron Co.,** *Chandler,* 1913
9. **Brazoria Co.,** *Palmer,* 1914
10. **Jefferson Co.,** *Palmer*, 1917

Clearly, *Heliotropium procumbens* was widely distributed early on in the state, this presumably reflective of a long history in the region. It is likely that some of the earlier professional collectors in the area, such as Berlandier (1828-1834) and Drummond (1833-1835), failed to collect the species because of its relatively restricted, edaphic, proclivities (heavy soils along waterways). In short, we conclude that

the species concerned is native to the state of Texas, and possibly coastal Louisiana.

ACKNOWLEDGEMENTS

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Ward, D.B. and P.R. Fantz. 1977. Keys to the Flora of Florida 3, Boraginacae. Phytologia 36: 309-323.
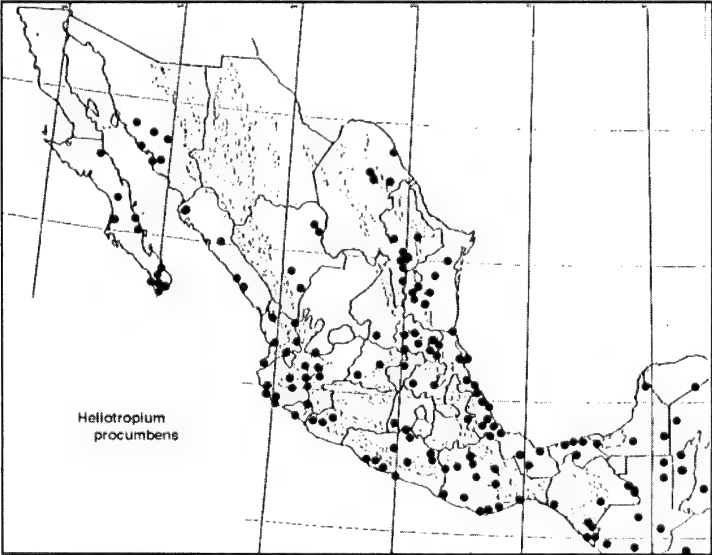
Fig. 1. Distribution of *Heliotropium procumbens* in Mexico (based upon specimens at LL-TEX and data on MEXU web pages).
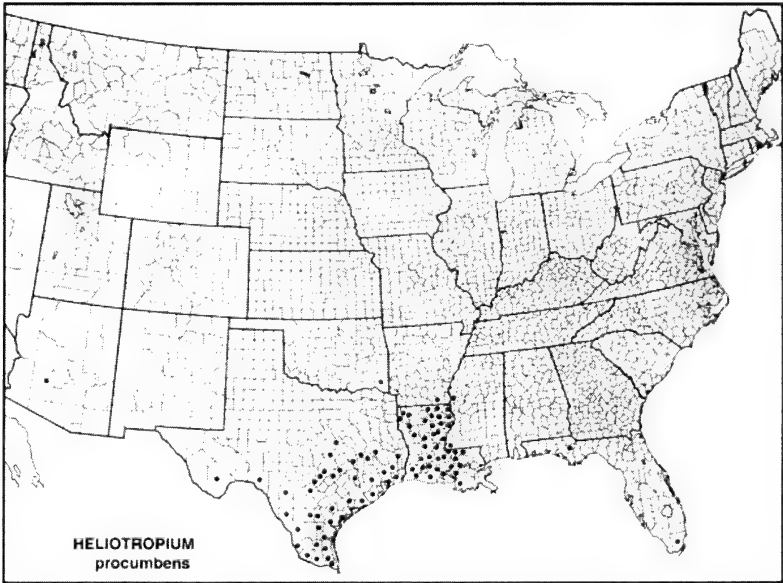
Fig. 2. Distribution of *Heliotropium procumbens* in the USA.
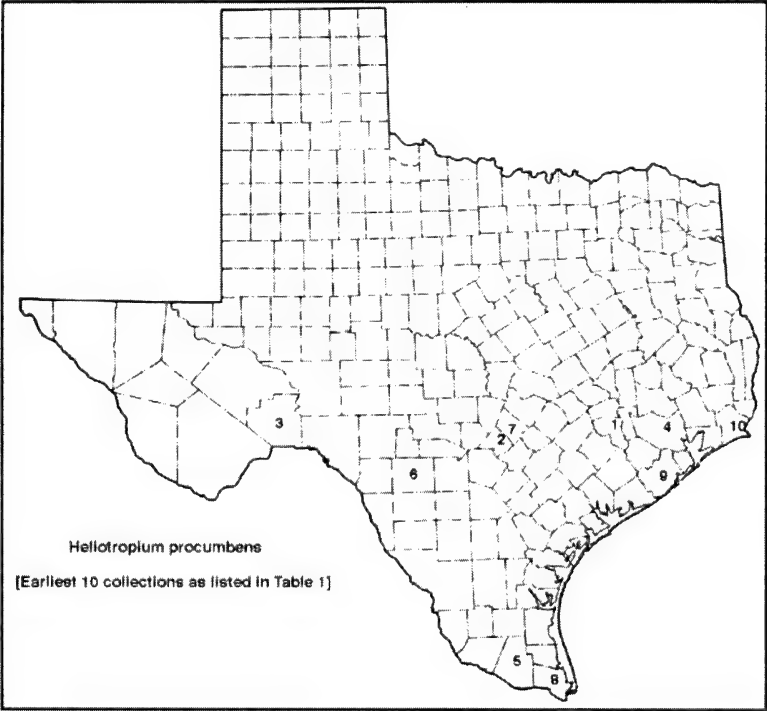
Fig. 3. Distribution of first 10 collections of *Heliotropium procumbens* in Texas, as listed in Table 1.

TAXONOMY OF *JUNIPERUS OXYCEDRUS* *VAR. SPILINANUS* IN TURKEY: LEAF TERPENOIDS AND SNPS FROM nrDNA AND petN

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ABSTRACT

Comparisons of SNPs of nrDNA and petN-psbM of *J. deltoides, J. oxycedrus,* and *J. o.* var. *spilinanus* revealed that *J. o.* var. spilinanus is allied with *J. deltoides* (Turkey), not *J. oxycedrus* (France and Spain). Leaf terpenoids showed the same pattern, supporting the recognition of *J. o.* var. *spilinanus* as **J. deltoides** R. P. Adams **var. spilinanus** (Yalt. et al.) Terzioğlu, **comb. nov.** *Phytologia 92(2): 156-166 (August 2, 2010).*

Recently, Yaltırık et al. (2007) described a new shrubby variety, *J. oxycedrus* L. var. *spilinanus* Yalt., Eliçin & Terzioğlu from Spildağı National Park of western Turkey. The occurrence of *J. oxycedrus* in Turkey seems probablematical as recent studies (Adams, 2004; Adams, et al., 2005) utilizing nrDNA sequencing, RAPDs, leaf

terpenoids and morphology, clearly indicate that *J. oxycedrus* (*sensu stricto*) is restricted to the western Mediterranean; another, sibling species, *J. deltoides* R. P. Adams occupies the eastern Mediterranean region, including Turkey (Fig. 1). The same pattern was found in nr
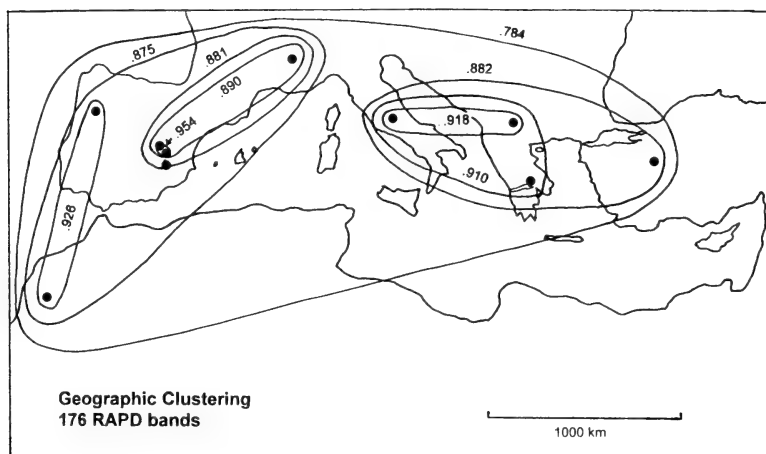
Figure 1. Differentiation of J. oxycedrus (left) and J. deltoides (right) based on 176 RAPD bands. (from Adams et al., 2005).

DNA sequences, leaf terpenoids and morphology (Adams et al., 2005). In his monograph of *Juniperus*, Adams (2008) recognized both *J. deltoides* and *J. oxycedrus*.

The purpose of the present study was to compare leaf terpenoids, SNPs from nrDNA and petN-psbM and morphology of the new *J. o.* var. *spilinanus* with *J. oxycedrus* (France, Spain) and *J. deltoides* (Turkey).

MATERIALS AND METHODS

Plant material: *J. deltoides, Adams 9430-9432*, Turkey; *J. oxycedrus*, *Adams 9039, 9040*, France, *9053* Spain; *J. o.* var. *spilinanus*, *Mataraci 1, 2, 3, (=Adams 10264-10266*; *=KATO 18791-18793)* Turkey. Voucher specimens are deposited at the Herbariums, Baylor University (BAYLU) and Karadeniz Technical University Faculty of Forestry (KATO).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing.   Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.).   Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational   measures   were   computed   using   absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principlal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Sequencing nrDNA revealed 12 nucleotide mutational events that included one mutation that occurred in only one individual among the taxa. The single nucleotide change was discarded from the SNPs leaving 11 nrDNA characters. A minimum spanning network based on the 11 SNPs is shown in figure 2 (left). No variation was found within

or among *J. deltoides*, *J. o.* var. *spilinanus* or *J. oxycedrus* (Fig. 2, left). However, *J .oxycedrus* (France, Spain) was separated by 11 nrDNA SNPs from *J. deltoides* (Turkey) and *J. o.* var. *spilinanus* (Fig. 2, left).
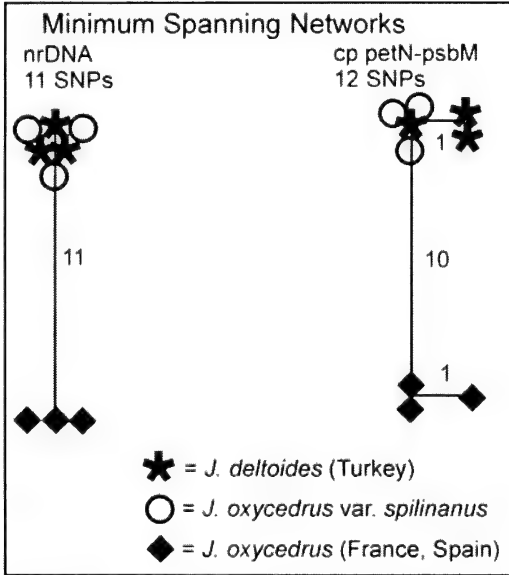
Figure 2. Minimum spanning networks based on nrDNA and petN-psbM SNPs. The numbers next to the lines are the number of SNPs.

Sequencing petN-psbM revealed 13 nucleotide mutational events that included a mutation that occurred in only one individual among the taxa. Discarding the singular event, resulted in 12 petN-psbM characters. *Juniperus oxycedrus* (France, Spain) was separated by 10 SNPs from *J. deltoides* - *J. o.* var. *spilinanus* (Fig. 2, right). A single SNP was found among *J. deltoides* and *J. oxycedrus*. It seems clear from nrDNA and petN-psbM data that *J. o.* var. *spilinanus* is conspecific with *J. deltoides*.

Leaf terpenoids

Overall, the leaf terpenoids of *J. oxycedrus* var. *spilinanus* are more like *J. deltoides* than *J. oxycedrus* (Table 1). Notice the concentrations of α-pinene, p-cymene, limonene, β-phellandrene, trans-pinocarveol, cis-p-menthal-2,8-dien-1-ol, myrtenal, carvone, (2E)-decenal, α-muurolene, α-calacorene, caryophyllene oxide, humulene epoxide II, and cadalene (Table 1).

The leaf terpenoids of *J. o.* var. *spilinanus* differ quantitatively from *J. deltoides* oil in the concentrations of myrcene (0.9, 3.8%) and δ-3-carene (0.1, 3.7%) and, based on this limited sampling, appear to have a few compounds not found in the oils of either *J. deltoides* or *J. oxycedrus*: ar-curcumene, trans-muurola-4(14), 5-diene, cubebol, and (E)-nerolidol.

Several of the diterpenoids are found in larger concentration in *J. o.* var. *spilinanus* than in *J. deltoides*, with amounts more similar to that of *J. oxycedrus*: manoyl oxide, abietatriene, abietadiene, and abieta-8(14),13(15)-diene (Table 1). However, the concentrations of these closely related diterpenes may be influenced by a few genes in that pathway, so one should be careful to not give too much weight to this similarity.

Morphology

Juniperus o. var. spilinanus is primarily separated from *J. oxycedrus* (Yaltirik et al., 2007) in being a shrub and having detached leaves with sagittate bases. The shape of detached leaf bases depends on how the leaf is attached to the stem. In *Juniperus,* sections *Juniperus* (approx. 12 species) and *Caryocedrus* (1 species) the awn-like leaves are attached directly to the stem and exfoliate by an abscission layer at the base of the leaf. In contrast, in *Juniperus* section *Sabina* (approx. 60 species with scale-like leaves) the leaf base clasps the stem and is attached to the stem. The shape of detached leaf bases has not previously been utilized in *Juniperus* taxonomy before Yaltirik et al. (2007). Examination of leaf bases of *J. oxycedrus* (sensu stricto), *J. o.* var. *spilinanus* and *J. deltoides* on leaves detached from herbarium specimens revealed that all three taxa have very similar detached leaf

bases. All of the detached leaf bases examined varied from a blunt to sagittate shape for the brown pre-abscission layer. Generally, the detached leaf base had a semi-circle shape that gave a sagittate shape. Both *J. deltoides* and *J. o.* var. *spilinanus* had leaves that scarcely narrowed at the base of attachment, in contrast to *J. oxycedrus* (France, Spain) that had leaves that narrowed at the base. In addition, both *J. deltoides* and *J. o.* var. *spilinanus* had stomatal bands scarcely sunken, whereas J. oxycedrus (*sensu stricto*) had sunken stomatal bands. Both *J. deltoides* and *J. o.* var. *spilinanus* had seed cones with raised cone scale tips, compared to seed cones globose, without raised cone scale tips for *J. oxycedrus*. *Juniperus o.* var. *spilinanus* fits well with *J. deltoides* in the European *Juniperus* key of Adams (p. 85, 2008).

In view of the data presented in this study, it is apparent that *J. o.* var. *spilinanus* is not related to *J. oxycedrus* (*sensu stricto*), but to *J. deltoides*. To reflect this evolutionary relationship, *J. o.* var. *spilinanus* is recognized as:

J. deltoides var. spilinanus (Yalt. et al.) Terzioğlu, **comb. nov.**
    Basionym: *Juniperus oxycedrus* L. subsp. *oxycedrus* var. *spilinanus* Yalt. et al., Turk. J. Bot. 31: 38, 2007. Type: Spildağı National Park, Turkey.

Distribution: The taxon is known from the type (KATO 13371) and Isotype (KTUB 533); Turkey, Manisa: Spildağı National Park, in *Pinus brutia* Ten. forest *P. nigra* J.F. Arnold subsp. *nigra* var. *caramanica* (Loudon) Rehder forests, on stony North and South slopes, 800-1400 m a.s.l. The specimens analyzed were collected by T. Mataracı, ibid (Adams 10264-10266 and KATO 18791 - 18793).

Table 1. Comparisons of the per cent total oil for leaf oils components of *J. oxycedrus*, France, *J. deltoides*, Turkey and *J. oxycedrus* var. *spilinanus*, shrubs, Turkey. Components that tend to separate the populations are highlighted in boldface. AI = Retention Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

AI	Compound	oxy	spil	delt
802	hexanal	t	0.1	0.6
855	(E)-2-hexenal	0.4	0.3	0.9
927	tricyclene	0.1	0.2	0.1
930	α-thujene	t	0.1	t
939	**α-pinene**	**53.2**	**34.1**	**32.7**
953	α-fenchene	0.1	t	0.3
954	camphene	0.6	0.3	0.6
960	thuja-2,4(10)-diene	t	0.6	0.4
975	sabinene	0.5	1.3	0.2
979	1-octen-3-ol	0.1	-	-
979	β-pinene	2.1	0.8	3.0
991	**myrcene**	**2.8**	**0.9**	**3.8**
1002	δ-2-carene	t	0.3	0.9
1003	α-phellandrene	t	1.1	1.8
1011	**δ-3-carene**	**5.1**	**0.1**	**3.7**
1017	α -terpinene	t	0.2	0.1
1025	**p-cymene**	**0.3**	**2.6**	**2.3**
1029	**limonene**	**3.5**	**6.4**	**6.0**
1030	**β-phellandrene**	**0.8**	**10.5**	**11.5**
1050	(E)-β-ocimene	t	t	-
1060	γ-terpinene	0.1	0.2	0.2
1070	cis-sabinene hydrate	-	0.1	-
1089	terpinolene	0.7	0.8	2.0
1099	linalool	t	-	0.7
1101	n-nonanal	t	0.1	0.5
1122	cis-p-menth-2-en-1-ol	-	0.4	0.3
1123	**trans-p-mentha-2,8-dien-1-ol**	**-**	**-**	**t**
1126	α -campholenal	0.8	1.2	1.3
1126	chrysanthenone	-	t	t
1137	**trans-pinocarveol**	**0.4**	**1.0**	**1.3**

AI	Compound	oxy	spil	delt
1138	**cis-p-mentha-2,8-dien-1-ol**	-	**0.2**	**0.1**
1141	cis-verbenol	t	0.4	0.4
1145	trans-verbenol	0.6	3.0	1.8
1163	trans-pinocamphone	-	-	0.1
1165	pinocarvone	t	0.3	0.6
1170	p-mentha-1,5-dien-8-ol	0.5	0.6	1.1
1175	cis-pinocamphone	-	-	0.1
1177	terpinen-4-ol	0.3	0.4	0.6
1181	naphthalene	0.1	-	0.3
1183	p-cymen-8-ol	t	0.6	1.0
1189	α -terpineol	0.6	0.3	1.2
1196	**myrtenal**	**t**	**0.6**	**0.6**
1205	verbenone	0.3	0.7	0.7
1217	trans-carveol	0.1	0.9	0.5
1229	**cis-carveol**	-	**0.2**	**t**
1242	cumin aldehyde	-	0.1	0.1
1243	**carvone**	-	**0.6**	**0.3**
1253	piperitone	-	0.2	t
1257	linalyl acetate	0.3	-	t
1264	**(2E)-decenal**	-	**0.1**	**0.2**
1289	bornyl acetate	0.7	0.5	0.9
1298	trans-pinocarvyl acetate	-	-	0.1
1298	carvacrol	-	0.2	t
1299	**(2E,4Z)-decadienal**	-	-	**0.4**
1317	**(2E,4E)-decadienal**	**0.1**	-	**0.8**
1342	trans-carvyl acetate	-	0.1	t
1346	trans-piperitol acetate	-	-	-
1349	**α-terpinyl acetate**	**0.2**	-	-
1373	α-ylangene	-	t	-
1377	**α-copaene**	-	-	**0.2**
1381	geranyl acetate	-	-	t
1388	β-bourbenene	0.3	0.2	0.2
1408	longifolene	-	-	0.6
1419	(E)-caryophyllene	0.4	0.7	1.2
1431	cis-thujopsene	-	-	0.1
1455	α-humulene	0.3	0.5	0.8
1480	γ-muurolene	0.1	-	t

AI	Compound	oxy	spil	delt
1485	germacrene D	2.3	-	0.7
1486	**ar-curcumene**	-	**0.2**	-
1494	**trans-muurola-4(14),5-diene**	-	**0.1**	-
1496	**2-tridecanone**	**0.3**	-	-
1500	**α-muurolene**	-	**1.1**	**0.4**
1514	γ-cadinene	0.7	0.5	0.4
1514	**cubebol**	-	**0.2**	-
1523	δ-cadinene	0.4	0.8	0.4
1541	**α-copaen-11-ol**	-	**0.2**	**0.1**
1546	**α-calacorene**	-	**0.5**	**0.5**
1561	germacrene B	0.1	-	-
1566	**β-calacorene**	-	-	**0.3**
1563	**(E)-nerolidol**	-	**1.2**	-
1567	dodecanoic acid	0.4	-	-
1583	**caryophyllene oxide**	**0.4**	**3.9**	**3.2**
1595	**salvial-4(14)-en-1-one**	**0.4**	-	-
1600	**hexadecane**	**0.3**	-	-
1601	cedrol	t	t	0.1
1608	**humulene epoxide II**	**0.3**	**1.7**	**1.1**
1619	**sesquiterpene alcohol, M+226**	**0.3**	-	-
1627	1-epi-cubenol	-	0.2	0.1
1640	epi-α-cadinol	0.6	0.1	-
1651	β-eudesmol	t	-	-
1654	**α-cadinol**	**1.6**	-	-
1661	cis-calamenen-10-ol	-	0.2	-
1674	**C10-dienol acetate, M+224**	**1.6**	-	-
1677	**cadalene**	-	**0.2**	**0.1**
1686	**germacra-4(15),5,10(14)-triene-1-al**	**1.6**	-	-
1700	**heptadecane**	**0.3**	-	-
1717	**(2E, 6E)-farnesol**	**0.3**	-	-
1725	**(E,E)-farnesol**	**0.6**	-	-
1746	**(2E, 6Z)-farnesol**	**0.4**	-	-
1790	**1-octadecene**	**t**	-	-
1800	**octadecane**	**t**	-	-
1807	**nootkatone**	**0.1**	-	-
1900	**nonadecane**	**0.1**	-	-

AI	Compound	oxy	spil	delt
1966	**sandaracopimara-8(14),15-diene**	**0.1**	**-**	**-**
1998	**manoyl oxide**	**6.2**	**5.5**	**1.3**
2014	palustradiene (=abieta-8,13-diene)	-	0.2	-
2017	**epi-13-manoyl oxide**	**0.1**	**-**	**-**
2023	abieta-8,12-diene	0.1	0.3	-
2057	**abietatriene**	**1.2**	**1.4**	**0.1**
2088	**abietadiene**	**1.3**	**3.4**	**-**
2154	**abieta-8(14),13(15)-diene**	**0.2**	**0.2**	**-**
2185	**sandaracopimarinal**	**0.2**	**-**	**-**
2190	**1-docosene**	**0.1**	**-**	**-**
2200	**docosane**	**0.1**	**-**	**-**
2218	**phytol acetate**	**0.1**	**-**	**-**
2300	**tricosane**	**0.2**	**-**	**-**
2312	abieta-7,13-dien-3-one	-	0.1	-

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GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS GRANDIS* (CUPRESSACEAE) AND COMPARISION WITH *J. OCCIDENTALIS* AND *J. OSTEOSPERMA*

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ABSTRACT

The leaf essential oils of *Juniperus grandis* were examined from throughout its range. The oil of the Yolla Bolly Mtns. putative *J. grandis* population was found to be most similar to *J. occidentalis*. The disjunct San Bernardino Mtns., *J. grandis* population was found to be quite differentiated from *J. grandis* populations in the high Sierras of California. The oils from several populations of *J. occidentalis* and *J. osteosperma,* as well as the oils of *J. californica* (chemotypes A and B), are compared with *J. grandis*. The compositions of leaf essential oils of *J. grandis*, *J. occidentalis* and *J. osteosperma* are presented in detail. *Phytologia 92(2): 167-185 (August 2, 2010).*

KEY WORDS: *Juniperus grandis (= J. occidentalis* var. *australis), J. californica, J. occidentalis, J. osteosperma,* Cupressaceae, terpenes, geographic variation.

Juniperus grandis R. P. Adams (= *J. occidentalis var. australis* (Vasek) A. & N. Holmgren) is part of a complex of closely related serrate leaf-margined junipers (*J. californica* Carr., *J. occidentalis* Hook., *J. osteosperma* [Torr.] Little) of the western United States and Baja Calif., Mexico. Vasek (1966), in a classic study of *J. californica, J. occidentalis* and *J. osteosperma*, recognized a new variety of *J. occidentalis* (*J. o.* var. *australis*). Additional research

utilizing leaf essential oils (Vasek and Scora, 1967) supported the new variety as well as the discovery of two chemical races of *J. californica* (chemotypes A and B).

Recently, DNA sequencing of nrDNA and trnC-trnD (Adams, et al., 2006) has shed new light on the relationships within this group. Firstly, the one-seeded, serrate leaf margined junipers were found to be paraphyletic. Secondly, *J. californica* was shown to be quite distinct (Fig. 1); however, analysis of nrDNA and trnC-trnD sequence data individually gives weak support that *J. californica* is sister to the *J. occidentalis - J. osteosperma* clade. Additional research will be needed to resolve this issue. Thirdly, all of the remaining species are divided into two large clades (Fig. 1), with *J. grandis* in a well-supported clade with *J. osteosperma*.
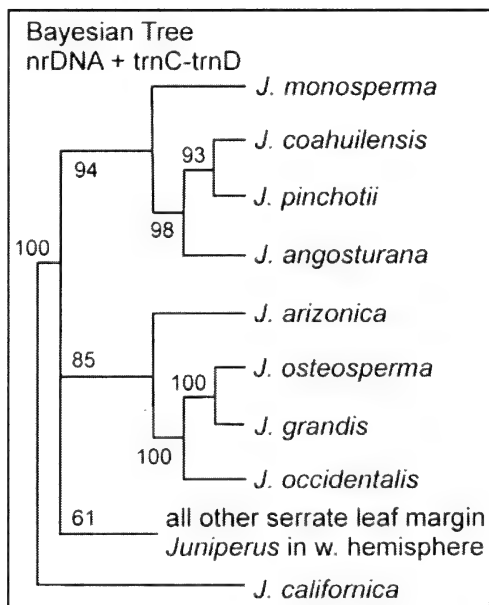
Figure 1. Partial phylogenetic tree derived from nrDNA + trnC-trnD sequence data (adapted from Adams et al., 2006). Values at the branch points are posterior probabilities.

Juniperus grandis has a major disjunction in its distribution, with populations in the high Sierras and the San Bernardino Mtns. (Fig. 2), and, according to Vasek (1966), with putative outlying
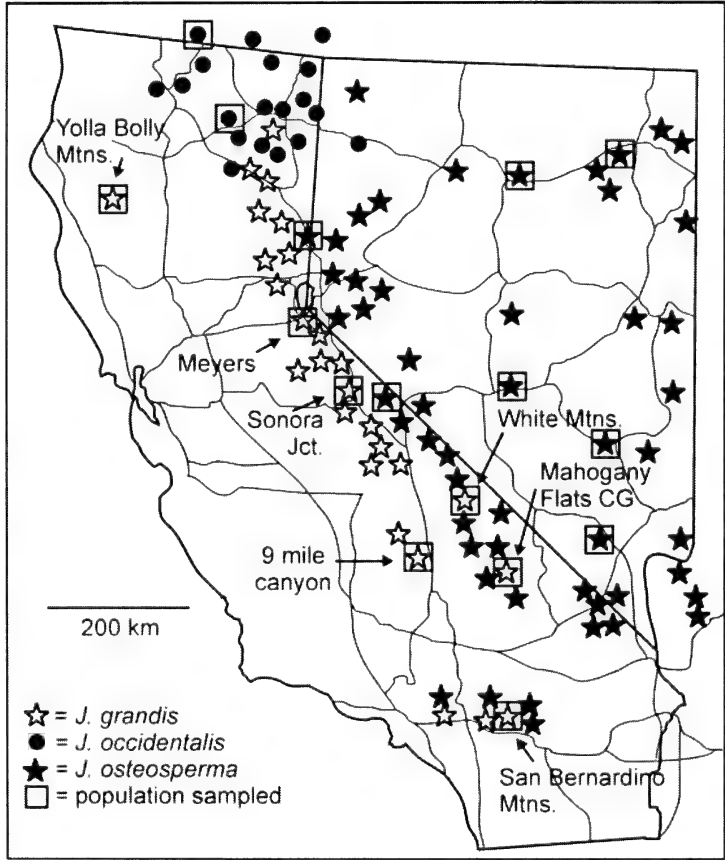
Figure 2. Distribution of *J. grandis* as per Vasek (1966) with populations sampled. Partial distributions of *J. occidentalis* and *J. osteosperma* are also mapped for this region. The putative (open stars) *J. grandis* populations (as per Vasek, 1966): Yolla Bolly Mtns., White Mtns. and Mahogany Flats represent the understanding prior to the present study, not the distribution of *J. grandis* as indicated by the terpene data in the present study.

populations in the Yolla Bolly Mtns., White Mtns., and Panamint Range (see Mahogany Flats CG, Fig. 2).

The leaf essential oils of *J. grandis* have been reported (as *J. occidentalis* var. *australis*) by Adams et al. (1983) and Adams (2000). However, both of these reports utilized samples taken only from the San Bernardino Mtns. Nothing has been published concerning geographic variation in the leaf essential oils of *J. grandis*.

The purpose of the present study is to present analyses of leaf essential oils of *J. grandis* from several populations and compare these with closely related species (*J. occidentalis, J. osteosperma*).

MATERIALS AND METHODS

Plant material:
J. californica, chemotype *A, Adams 10154-10156,* Victorville, CA, *Adams 8695-8697*, 13 km n of Amboy/Kelso exit on I40, on road to Kelso at Granite Pass, 34º 48.41N, 115º 36.54'W, 1280 m, San Bernardino Co., CA; *J. californica,* chemotype B *Adams 8698-8699*, 27 km se of SE of Yucca, on Alamo Road, 34º 44.91N, 113º 58.19'W, 920 m, Mojave Co., AZ;
J. grandis, Adams 11963-11967, Jct. US 50 & CA 89, 38º 51.086N, 120º 01.244'W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38º 18.289'N, 111º 35.598'W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35º 54.003'N, 118º 02.078'W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34º 17.533'N, 116º 49.153'W, 2053 m, San Bernardino Co., CA;
J. occidentalis, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45º 44.392'N, 120º 41.207'W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44º 53.676'N, 120º 56.131'W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44º 02.390'N, 121º 20.054'W, 1132 m, Deschutes Co., OR; *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43º 53.922'N, 120º 59.187'W, 1274 m, Deschutes Co., OR; *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR66, 42º 08.044'N, 122º

34.130'W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m, Lassen Co., CA; *Adams 11995-11998 (Kauffmann A1-A3, B1),* Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA;

J. osteosperma, Adams 10272-10276, on NV157, Charleston Mtns. 36° 16.246'N, 115° 32.604'W, 1795 m, Clark Co., NV; *Adams 11122-11124*, Hancock Summit, mile 38 on US375, 37° 26.404'N, 115° 22.703'W, 1675 m, Lincoln Co. NV; *Adams 11125-11127*, McKinney Tanks Summit on US6, 38° 07.005'N, 116° 54.103'W, 1933 m, Nye Co., NV; *Adams 11134-36*, 8 km s of Bridgeport, on US395, 38° 12.639'N, 119° 13.846'W, 2004 m, Mono Co., CA; *Adams 11141-11143,* 13 km w of Elko, on I80, 40° 45.598'N, 115° 55.942'W, 1535 m, Elko Co., NV; *Adams 11144-11146*, 8 km e of Wells, on I80, 41° 06.533'N, 114° 51.441'W, 1876 m, Elko Co., NV; *Adams 11960-11962,* 56 km n of Reno, NV; on US395, 39° 54.458'N, 120° 00.322'W, 1383 m, Lassen Co., CA; *Adams 11973-11977,* 10 km n of CA 168 on White Mtn. Rd., 37° 20.143'N, 118° 11.346'W, 2607 m, Inyo Co., CA; *Adams 11978-11982,* Mahogany Flats Campground, Panamint Mtns., 36° 13.783'N, 117° 04.102'W, 2477 m, Inyo Co., CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron

coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

A minimum spanning network based, on 63 terpenes, revealed (Fig. 3) the taxa to be aligned in five groups: *J. osteosperma, J. californica, J. occidentalis, J. grandis* (San Bernardino Mtns.) and *J. grandis* (high Sierras). *Juniperus osteosperma* is the most uniform taxon, even though it includes populations that were putatively *J. grandis* (White Mtns, CA and Mahogany Flats campground, Panamint Mtns., CA). In figure 2, one can see that Vasek (1966) called plants from these areas *J. grandis.* Two *J. grandis* (filed as *J. occidentalis* var. *australis*) herbarium specimens were found from the White Mtns./ Inyo Mtns: UCR4254, *Vasek, Clarke & Kucera 650710-08*, 10 Jul 1965, Inyo Mtns., Seep Hole Springs (2800 m) near Waucoba Saddle, large trees with red bark; UCR99917, *G. K. Helmkamp 2460*, 30 Sept 1997, White Mtns., 6.1 mi. n of CA168 on White Mtn. Rd., 2622 m, abundant on steep rocky bank. Our population, 6.2 mi. n of CA168 on White Mtn. Rd., is very near the site of Helmkamp. The trees had a tendency to have a single axis, but were somewhat branched. Clearly, from the leaf oils (Fig. 3), our samples were typical oils of *J. osteosperma.* Of course, it may be that there are some *J. grandis* in the White Mtns. that were not sampled in this limited collection. I (RPA) was not able to visit Seep Hole Springs to collect from the Vasek et al. site, but the site seems to be in a micro-habitat at 2800 m and may well be a stand of *J. grandis* isolated from the high Sierra populations.

Two *J. grandis* (filed as *J. occidentalis* var. *australis*) specimens were found from the Panamint Mtns.: UCR1808, *Vasek 610909-06*, 09 Sept 1962, ca. 200 yds. s of Mahogany Flat, 2561 m and UCR 1812 *Vasek 620930-01*, 30 Sept 1962, s of Mahogany Flat, just below the new Mt. Rogers Rd., 2500 m. This area was visited (RPA) and appears to be where present day Mahogany Flat campground (CG)

is located (2477 m). Several *J. grandis - osteosperma* trees were seen that had a strong central axis and the oils from these were extracted and examined. All had typical *J. osteosperma* oils (Fig. 3). This site is more mesic than the lower desert area where smaller, multi-stemmed
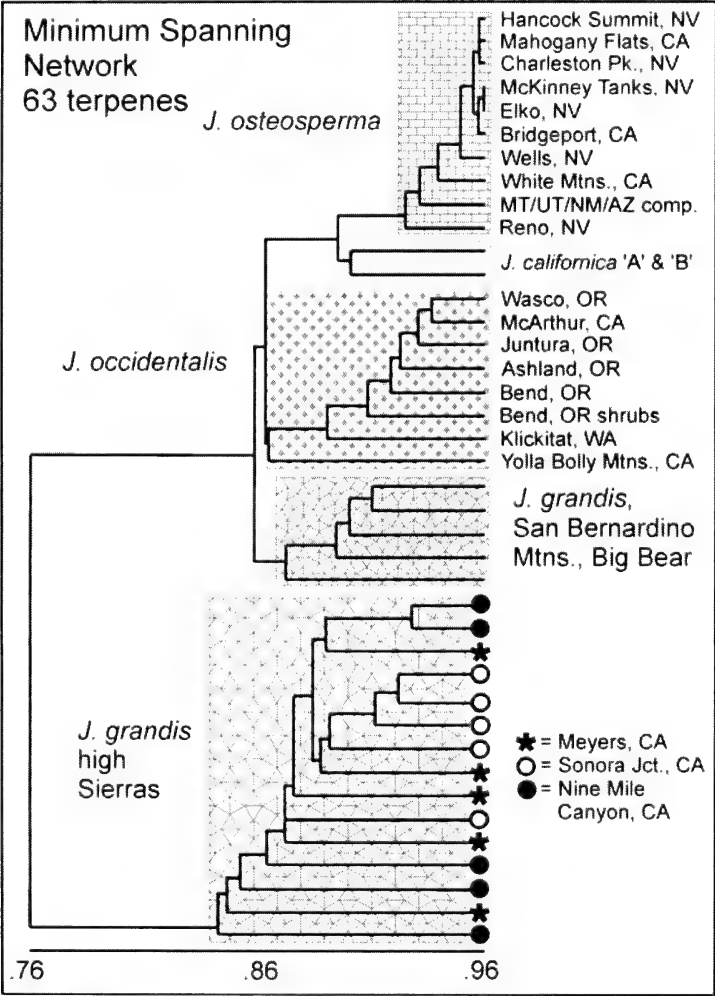
Figure 3. Minimum Spanning Network based on 63 terpenes.

J. osteosperma grow in profusion. It appears that when *J. osteosperma* grows in a more mesic site, it becomes more tree-like and as a result it may be confused with *J. grandis*.   However, *J. grandis* has a characteristic trunk shape that (tapered from the base to the top of the tree). In older trees in the high Sierras such trunks are quite noticeable.

The putative *J. grandis* trees in the Yolla Bolly-Middle Eel Wilderness (Fig. 2), grow on sandy soil with Jeffery pine, sugar pine, Douglas fir, incense cedar and mountain mahogany at 1815- 2000 m. This site is very isolated from the high Sierra *J. grandis* populations and is in a quite mesic environment. The oils from the Yolla Bolly Mtns. junipers were most similar to the Juntura, OR and McArthur, CA *J. occidentalis* populations (links not shown in Fig. 3).

The major trend in figure 3 is the splitting of *J. grandis* into the high Sierras populations and San Bernardino Mtns. population. In fact, the oils of *J. grandis* from the San Bernardino Mtns. are more similar to *J. occidentalis, J. osteosperma* and *J. californica* than to the oils of the high Sierras populations. Notice, that among the three high Sierra populations, there appears to be little differentiation between the Meyers, Sonora Jct., and Nine Mile Canyon populations, as the individuals are interspersed in the cluster.

To further examine these groupings, Principal Coordinates Ordination (PCO) was performed using 63 terpenes.   Factoring the similarity matrix resulted in eigenroots that appeared to asymptote after the first four eigenroots. These eigenroots accounted for 32.2, 12.46, 7.91 and 4.03 % of the variance among individuals. Ordination shows (Fig. 4) the five major groups. The oils of *J. grandis* from the San Bernardino Mtns. appear to be fairly similar to the oils of *J. occidentalis*.   However, they were further separated from *J. occidentalis* by the fourth eigenroot (4.03%) (not shown in figure 4).

PCO of the 20 *J. grandis* individuals, plus *J. occidentalis* from McArthur, CA and Yolla Bolly Mtns., CA resulted in four eigenroots before they began to asymptote. These four eigenroots accounted for 35.05, 7.89, 6.40 and 5.89% of the variance. Ordination of the *J. grandis* individuals plus population averages from McArthur and Yolla Bolly Mtns., CA, shows (figure 5) the two groups of *J. grandis* (San

Bernardino Mtns. and high Sierras populations) plus *J. occidentalis* McArthur and Yolla Bolly Mtns. populations. Notice that the similarity between McArthur and Yolla Bolly Mtns. (0.82) is only slightly larger than between Yolla Bolly Mtns. and *J. grandis*, San Bernardino Mtns. (0.79), but much larger than the link to *J. grandis*, high Sierras (0.71). It should be noted that the Yolla Bolly Mtns. population is the most differentiated population of *J. occidentalis* (figure 3).
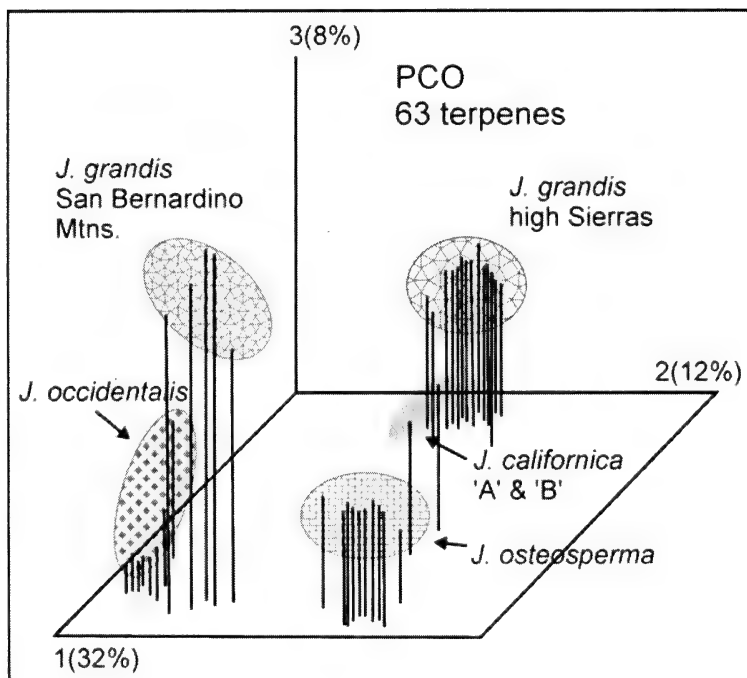
Figure 4. PCO based on 63 terpenes. See text for discussion.

Table 1 shows the leaf oil compositions for the four *J. grandis* populations and representative oils of *J. osteosperma* and *J. occidentalis*. The *J. grandis* (high Sierras vs. San Bernardino Mtns.) populations differ in many compounds: α-fenchene (1.4-1.5%, 0.2);
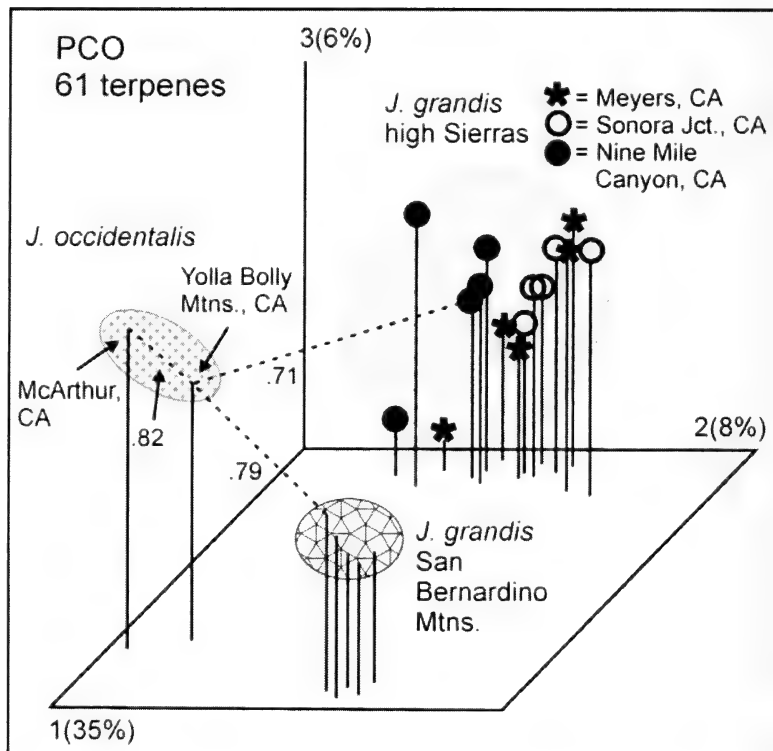
Figure 5. PCO based on 61 terpenes from *J. grandis* (20 individuals) and *J. occidentalis* populations (McArthur, Yolla Bolly Mtns., CA). The dotted lines are minimum links that connect the groups. The numbers by the dotted lines are the similarity (0.0 - 1.0 scale).

verbenene (1.7-2.9, 0.3), sabinene (0-trace, 24.3), α-phellandrene (1.3-2.3, 0.4), δ-3-carene (17.9-30.0, 2.8), p-cymene (1.4-1.6, 6.5), β-phellandrene (10.3-16.4, 1.5), γ-terpene (0.2-0.3, 4.9), cis-sabinene hydrate (0, 1.9), unknown 1092 (0.9-1.2, 0), trans-sabinene hydrate (0, 1.8), camphor (0, 1.2), neo-isopulegol (0.5-1.1, 0), sabina ketone (0, 0.9), terpinen-4-ol (0.4, 9.3), coahuilensol, methyl ether (0.4-1.8, 0), unknown 1230 (2.3-3.9, 0.4), piperitone (1.2-3.6, 0), methyl geranate (0, 1.8), trans-calamenene (0, 2.3), δ-cadinene (0.8-1.3, 0), elemol (0-trace, 0.9), germacrene-D-4-ol (0.7, 0) and α-eudesmol (0, 0.6). The oil

of the high Sierra *J. grandis* is dominated by δ-3-carene (17.9-30.0%) whereas the oil of the San Bernardino Mtns. population is dominated by sabinene (24.3%).

The percentages of several compounds of *J. grandis*, San Bernardino Mtns., are similar to *J. osteosperma* and *J. occidentalis* (Table 1): sabinene, camphene, myrcene, α-phellandrene, β-phellandrene, γ-terpene, cis- and trans-sabinene hydrate, sabina ketone, and terpinen-4-ol. It is easy to see why *J. grandis*, San Bernardino Mtns., oil was more similar to *J. occidentalis* and *J. osteosperma* than to *J. grandis* from the high Sierras (figure 3).

Leaf essential oils are extremely useful for the analyses of populational differentiation, hybridization and introgression and in assigning individual plants to a species. It is clear from the present study that some plants (Panamint Mtns. and White Mtns.) that resemble *J. grandis* are actually large, single stemmed *J. osteosperma* plants. The Yolla Bolly Mtns. upright junipers appear to be part of *J. occidentalis,* not *J. grandis*.

Terpenes are generally not as useful in making phylogenetic decisions because several terpenes may be controlled by a single enzyme (ex. a terpene alcohol synthase might add OH to several kinds of terpenes). Thus, adding up the number of terpene differences may or may not give a good estimation of divergence. However, the number and scope of terpene differences between the San Bernardino Mtns. and high Sierra *J. grandis* populations indicate considerable differentiation. Additional research, using DNA sequencing, should help in elucidating these relationships.

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for four populations of *J. grandis*, (Meyers, CA; 16 km w of Sonora Jct., CA; 9 Mile Canyon, CA and Big Bear City, CA) plus *J. osteosperma* (McKinney Tanks, NV) and *J. occidentalis* (Bend, OR). Compounds in boldface appear to separate taxa and were used in numerical analyses.

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
921	**tricyclene**	-	-	0.3	0.3	0.8	1.1
924	**α-thujene**	-	-	-	2.3	0.5	1.0
932	**α-pinene**	14.0	7.3	12.0	7.1	4.4	5.0
945	**α-fenchene**	1.5	1.4	1.5	0.2	-	t
946	**camphene**	-	-	-	0.3	1.1	1.0
953	thuja-2,4-diene	t	t	t	-	t	t
961	**verbenene**	2.9	1.7	2.5	0.3	-	-
969	**sabinene**	-	t	t	24.3	10.2	12.0
974	**β-pinene**	1.3	0.6	1.0	0.5	0.2	0.4
988	**myrcene**	3.1	3.5	2.9	1.7	1.7	1.3
1001	**δ-2-carene**	1.1	0.2	0.8	0.1	-	t
1002	**α-phellandrene**	1.6	2.3	1.3	0.4	0.3	0.8
1008	**δ-3-carene**	27.3	30.0	17.9	2.8	-	1.0
1014	**α-terpinene**	0.4	0.4	0.1	3.0	1.3	1.7
1020	**p-cymene**	1.4	1.4	1.6	6.5	2.4	10.7
1024	**limonene**	1.2	1.8	1.2	1.6	2.1	0.9

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1025	**β-phellandrene**	**10.6**	**16.4**	**10.3**	**1.5**	**3.2**	**3.5**
1044	(E)-β-ocimene	t	t	0.2	0.3	t	0.1
1054	**γ-terpinene**	**0.3**	**0.2**	**0.3**	**4.9**	**2.1**	**3.0**
1065	**cis-sabinene hydrate**	-	-	-	**1.9**	**0.8**	**0.9**
1078	camphenilone	-	-	-	-	t	-
1086	**terpinolene**	**3.7**	**3.7**	**3.3**	**1.9**	**1.4**	**1.3**
1090	6,7-epoxymycene	-	-	-	-	0.1	-
1092	**96, 109,43,152, C10-OH**	**0.9**	**0.9**	**1.2**	-	-	-
1095	linalool	t	0.2	0.4	-	-	0.5
1098	**trans-sabinene hydrate**	-	-	-	**1.8**	**1.0**	**0.7**
1100	55,83,110,156, unknown	-	-	-	-	-	0.3
1102	isopentyl-isovalerate	-	-	-	-	0.2	-
1112	3-me-3-buten-methyl butanoate	-	-	-	-	0.4	-
1112	trans-thujone	-	-	-	0.2	-	t
1118	cis-p-menth-2-en-1-ol	0.8	1.2	0.6	0.7	0.6	0.7
1122	α-campholenal	t	t	t	-	0.3	-
1132	cis-limonene oxide (furanoid)	t	t	t	-	-	-
1136	**trans-p-menth-2-en-1-ol**	**0.9**	**1.0**	**0.7**	**0.8**	-	**0.9**
1141	**camphor**	-	-	-	**1.2**	**23.7**	**2.5**
1144	**neo-isopulegol**	**0.5**	**0.8**	**1.1**	-	-	-
1145	**camphene hydrate**	t	t	**0.2**	**0.2**	**1.5**	**0.2**

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1154	**p-menth-1,5-dien-8-ol iso.**	**0.6**	**0.8**	**0.7**	-	-	-
1154	**sabina ketone**	-	-	-	**0.9**	**0.8**	**0.4**
1161	**p-menth-1,5-dien-8-ol iso.**	**0.3**	**0.3**	**1.3**	**0.1**	-	-
1165	**borneol**	-	-	-	**0.1**	**6.0**	**2.2**
1166	**coahuilensol**	t	**0.3**	-	-	-	**0.6**
1174	**terpinen-4-ol**	**0.4**	**0.4**	**0.4**	**9.3**	**8.3**	**6.7**
1176	**m-cymen-9-ol**	**0.4**	**0.5**	**1.2**	-	-	-
1179	p-cymen-8-ol	0.4	0.5	1.0	1.0	0.5	0.5
1186	α-terpineol	1.2	1.8	1.1	0.3	0.4	0.4
1195	myrtenol	-	-	-	0.2	0.2	-
1195	cis-piperitol	0.4	0.3	0.2	0.2	0.3	0.2
1204	verbenone	-	-	-	-	0.2	-
1207	trans-piperitol	0.9	1.0	0.8	0.6	0.3	0.3
1215	trans-carveol	-	-	-	-	0.6	-
1219	**coahuilensol, me-ether**	**0.4**	**0.5**	**1.8**	-	**0.2**	**1.1**
1223	**citronellol**	t	**0.6**	**0.3**	**0.2**	**8.3**	**8.4**
1230	43,119,152,194, unknown	3.9	2.3	2.5	0.4	-	-
1238	**cumin aldehyde**	-	-	-	**0.3**	**0.3**	**0.2**
1239	carvone	t	t	t	-	0.6	-
1249	**piperitone**	**1.2**	**1.2**	**3.6**	-	t	**0.2**
1253	trans-sabinene hydrate ac	-	-	-	0.6	-	-
1254	linalool acetate	-	-	-	-	-	0.1

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1255	4Z-decenol	0.4	0.4	-	-	-	-
1257	methyl citronellate	0.2	0.4	-	0.1	-	-
1260	152,123,77,109, C10-OH	-	t	-	0.2	-	-
1274	**neo-isopulegyl acetate**	**0.3**	**1.4**	**0.2**	-	-	-
1283	α-terpinen-7-al	-	-	-	-	0.2	-
1284	**bornyl acetate**	**0.4**	**0.6**	**2.3**	**2.2**	**16.6**	**9.5**
1285	**safrole**	**0.3**	**0.5**	**2.3**	-	-	-
1298	carvacrol	0.2	0.2	0.4	0.2	t	0.4
1298	3'-methoxy-acetophenone	-	-	-	0.2	-	-
1319	**149,69,91,164, phenolic**	**0.8**	**0.7**	**3.2**	-	**0.4**	-
1322	**methyl-geranate**	-	-	-	**1.8**	-	**1.0**
1325	**p-mentha-1,4-dien-7-ol**	-	-	t	**0.7**	**0.5**	t
1332	**cis-piperitol acetate**	**0.4**	**0.2**	t	-	-	-
1343	trans-piperitol acetate	0.3	0.2	t	-	-	-
1345	α-cubebene	-	-	-	t	-	t
1350	citronellyl acetate	-	-	-	-	-	-
1374	α-copaene	-	-	-	-	-	-
1387	β-bourbonene	0.5	0.3	0.3	0.2	-	1.0
1387	β-cubebene	-	-	-	0.3	-	0.2
1388	**79,43,91,180, unknown**	**0.3**	**0.3**	**0.2**	-	-	-
1389	**111,81,151,182, unknown**	**1.0**	**0.9**	**0.9**	**0.4**	-	-
1403	methyl eugenol	t	0.2	t	-	-	-

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1417	(E)-caryophyllene	-	-	-	0.2	-	-
1429	**cis-thujopsene**	-	-	-	-	0.7	**0.9**
1448	cis-muurola-3,5-diene	t	t	-	0.2	-	-
1451	trans-muurola-3,5-diene	-	-	-	-	-	0.1
1452	α-humulene	-	-	-	-	-	-
1465	cis-muurola-4,5-diene	-	-	t	0.1	-	0.1
1468	**pinchotene acetate**	-	-	-	-	0.5	**0.6**
1471	<u>121,105,180,208,phenol</u>	**0.3**	**0.4**	**2.0**	**0.3**	-	-
1471	dauca-5,8-diene	-	-	-	0.2	-	0.3
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	0.3
1478	**γ-muurolene**	-	**t**	**t**	**0.2**	-	**0.8**
1484	germacrene D	0.2	0.2	t	0.3	-	0.3
1491	43,207,161,222, C15-OH	-	-	-	0.3	-	-
1493	trans-muurola-4(14),5-diene	-	-	-	-	-	-
1493	epi-cubebol	-	t	-	0.2	-	0.4
1500	α-muurolene	0.3	0.2	0.4	0.5	-	0.4
1513	**γ-cadinene**	**1.3**	**0.8**	**1.2**	-	t	**1.1**
1518	**epi-cubebol**	**0.4**	**0.4**	**1.1**	**1.2**	t	**3.7**
1521	**trans-calamenene**	-	-	-	1.5	-	**0.4**
1522	**δ-cadinene**	**1.1**	**0.8**	**1.3**	**2.3**	**0.2**	**4.1**
1533	trans-cadina-1,4-diene	-	-	-	0.1	-	0.1

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1537	α-cadinene	t	-	t	0.2	-	0.4
1544	α-calacorene	-	-	-	-	-	0.3
1548	**elemol**	-	**t**	-	**0.9**	**0.9**	-
1555	**elemicin**	**1.5**	**1.4**	-	-	-	-
1559	germacrene B	-	-	-	0.1	-	-
1561	**1-nor-bourbonanone**	-	-	-	**1.1**	-	-
1561	(E)-nerolidol	-	t	-	-	-	-
1574	**germacrene-D-4-ol**	**0.7**	**0.7**	**0.7**	-	**t**	**0.6**
1582	caryophyllene oxide	t	t	t	0.3	t	-
1586	gleenol	-	-	-	-	-	0.3
1587	trans-muurol-5-en-4-α-ol	-	-	-	t	-	-
1607	**β-oplopenone**	**0.4**	**0.3**	-	**0.8**	-	**0.4**
1608	humulene epoxide II	-	-	-	-	t	-
1618	1,10-di-epi-cubenol	t	t	-	-	-	0.2
1627	1-epi-cubenol	t	t	0.3	0.5	-	1.6
1630	γ-eudesmol	-	-	-	t	0.2	-
1638	**epi-α-cadinol**	**0.7**	**0.7**	**0.7**	**0.6**	**t**	**1.1**
1638	epi-α-muurolol	0.7	0.7	0.8	0.6	t	1.2
1644	α-muurolol	t	0.2	t	0.1	-	0.7
1649	β-eudesmol	0.4	t	-	0.2	0.2	-
1652	**α-eudesmol**	-	-	-	**0.6**	**0.2**	-
1652	**α-cadinol**	**1.6**	**1.4**	**1.7**	**0.7**	**0.2**	**1.8**

AI	Compound	grandis Meyers	grandis Son. Jct	grandis 9 mile	grandis Big Bear	osteo McKin	occid Bend
1670	bulnesol	-	-	-	-	t	-
1675	cadalene	-	-	-	0.1	-	0.3
1687	43,167,81,238, unknown	-	-	-	0.3	-	-
1688	shyobunol	0.2	0.2	t	-	-	-
1699	epi-nootkatol	-	t	t	-	-	-
1739	oplopanone	t	t	t	0.2	t	-
1987	**manoyl oxide**	**t**	**0.1**	**t**	**t**	**-**	**3.2**
2009	epi-13-manoyl oxide	-	-	-	-	-	t
2056	manool	t	-	t	-	-	-
2055	abietatriene	t	t	-	-	-	-
2298	4-epi-abietal	t	0.1	t	-	-	-
2312	abieta-7,13-dieen-3-one	-	-	-	-	0.1	-

AI = Arithmetic Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

CHEMOSYTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION

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ABSTRACT

The essential oils of leaves of *J. pinchotii* and *J. virginiana* tree were collected and analyzed as fresh vs. air dried then stored at ambient conditions (21° C) for various periods before extraction. The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of the 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but considerable intermixing of samples. Drying *J. virginiana* leaves under extreme conditions (fresh vs. 30° C, 60° C, 100° C) revealed considerable changes in the oils at the highest drying temperature (100° C) with the loss of more volatile monoterpenes, and a relative increase (on a % total oil basis) of the diterpenes. Surprisingly, there was little change in the oils between the fresh, 30° C and 60° C samples. Comparison of *J. pinchotii* fresh and dried (2 weeks) oils revealed 5 significant and 3 highly significant differences, but the overall profile was little changed. It appears one can use the oils from dried leaves of *Juniperus* for chemosystematic studies. *Phytologia 92(2): 186-198 (August 2, 2010).*

KEY WORDS: *Juniperus pinchotii, J. virginiana,* oils from dried leaves, chemosystematics.

When collecting and transporting plant specimens across international borders, one encounters considerable difficulty with government customs agents. The transport of silica gel dried leaves (for DNA analyses) is generally easy. The importation of fresh plant

materials is subject to plant quarantine laws. However, herbarium vouchers are generally (in the author's experience) permitted without too much difficulty. Part of the ease of importing herbarium specimens is because specimens are often frozen to kill insects, then air dried.

A second problem of using fresh leaves is that refrigeration in the field is often impossible and international air travel using dry ice is getting to be extremely difficult. The use of air dried leaves for essential oils would make the problems much more manageable.

Recently, Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22° C, 16 days) leaves for *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. The first two species are in section *Sabina* and have scale-leaves, whereas *J. oxycedrus* is in section *Juniperus* with awl-like leaves (Adams, 2008). They reported small to moderate changes in several components, however, no statistical data were published.

The purpose of this study was to determine if the changes in oil composition when using air dried leaves precludes their use in chemosystematics.

MATERIALS AND METHODS

Plant material - *J. pinchotii*, *Adams 11890*, 14.2 mi. s of Claude on TX 207, Armstrong Co., TX; *J. virginiana, Adams11768,* cultivated, nw corner of Gruver City Park, Hansford Co. TX, first sample for monthly drying test taken on 23 Apr 2009; 2nd sample for extreme drying tests taken on 10 May 2009 . Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g.) and air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oil of *J. virginiana* and a comparison of components over the 8 month storage period. The compounds are remarkably stable during the drying and storage tests. Only 9 compounds significantly differ: α-pinene, sabinene, β-phellandrene, terpinen-4-ol, γ-cadinene, δ-cadinene, elemol, germacrene D-4-ol and 4-epi-abietal. Four compounds differed highly significantly: pregeijerene B, safrole, methyl eugenol, and 8-α-acetoxyelemol. Interestingly, the per cent oil yield (on an oven dried, 100° C, 48h, wt. basis) did not vary significantly! One might expect that the more volatile monoterpenes (e.g., α-pinene, sabinene, limonene, β-phellandrene, etc.) would volatilize upon drying and storage, but in this instance the relative percentages were about the same after 8 mo. at 21° C as in the fresh leaves (Table 1). The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle (Fig. 1). So volatilization in this instance seems to be minimized by the intact glands and waxy cuticle.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (figure 1) shows that the samples do cluster somewhat by sample date, but there is considerable interspersion of samples. The first 9 eigenroots of the similarity matrix accounted for 14.7, 11.6, 11.0, 9.7, 7.38, 6.93, 5.62, 4.67 and 3.86% of the variance. It is obvious from the eigenroots (and the PCO, figure 2) that the oils from fresh leaves did not account for a large portion of the variance as one might have expected if large changes in composition occurred between the fresh leaves and the first and second weeks of drying.

J. virginiana |¹¹¹¹|¹¹¹¹| 1 mm

Figure 1. Leaves of *J. virginiana*.

Because the changes in composition from fresh to air dried leaves were so small, it seemed of interest to investigate the effects of extreme drying on oils. A second sample of *J. virginiana* leaves was collected (10 May 2009) and subjected to 4 treatments: fresh and dried at: 30° C (24h), 60° C (24h), and 100° C (24h). A comparison of these oils is shown in table 2. As one would expect, there was a significant decrease in the percent oil yields with increased drying temperatures (Table 2). Five (5) compounds varied significantly and twenty five (25) compounds varied highly significantly (Table 2). Several of the major compounds were stable even upon drying at 60° C, then decreased at 100° C: sabinene, limonene, β-phellandrene, pregeijerene B. The opposite trend (increased at 100° C) was found for: safrole, elemol and 8-α-acetoxyelemol (Table 2). Only one new compound was formed, cyclohexadecanolide (trace at 60° C, 0.5% at 100° C). The stability of compounds *in situ* even at extreme drying was surprising.
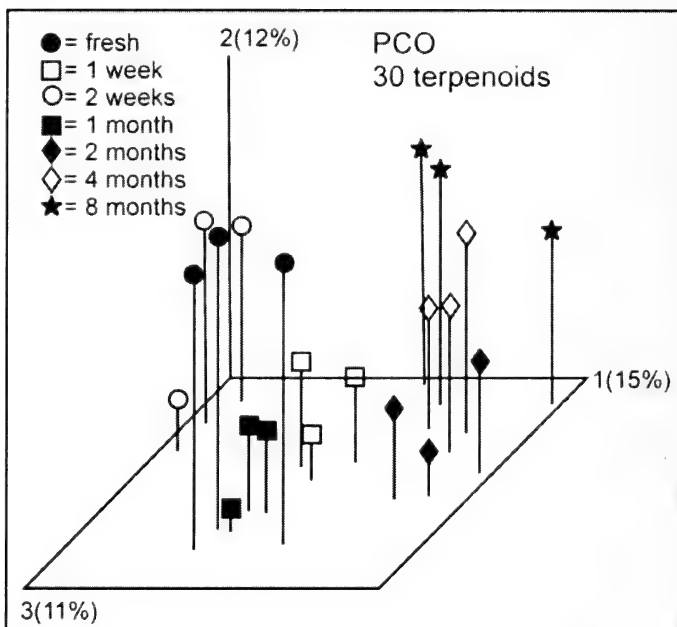
Figure 2. PCO based on 30 terpenoids of the oils from J. virginiana samples dried for periods of 1 week to 8 months at 21° C.

Juniperus pinchotii is in the serrate-leaf margined *Juniperus* group and has oil glands that rupture with white exudate on the leaves (Fig. 3). The white crystalline exudate is composed mostly of camphor and bornyl acetate (Adams, 2008). A comparison of the leaf oils of *J. pinchotii* from fresh vs. air dried (21° C, 2 weeks) leaves is shown in Table 3.
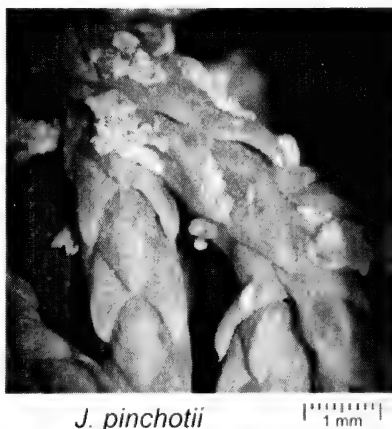
J. pinchotii

Figure 3. Exudate on *J. pinchotii*.

Surprisingly, the oil from fresh and dried leaves are very similar (Table 3). The largest volatile terpene, sabinene had a non-significant change upon leaf drying. However, the percent oil yield (oven dry wt. basis) showed a highly significant decline (Table 3) in contrast to *J. virginiana* that displayed no significant changes in percent oil yields during drying and storage (Table 1). For the *J. pinchotii* oils, only five compounds varied significantly: tricyclene, limonene, camphor, bornyl acetate and β-eudesmol and two components varied highly significantly: borneol and terpinen-4-ol (Table 3). In spite of the large decrease in oil yield with drying, the overall composition of *J. pinchotii* leaf oil remained relatively unchanged.

CONCLUSIONS

For *J. virginiana* and *J. pinchotii* (and other *Juniperus* species) the leaf oils appear to be sufficiently stable in dried leaves (21° C and 30° C) to justify their use in chemosystematic studies. For studies of infraspecific geographical variation, it appears that if all the samples were subjected to the same drying regime, the oils would be comparable. Chemosystematics among species with large differences in the essential oil compositions appear to be valid, even if oils from both fresh and air dried leaves were utilized.

ACKNOWLEDGEMENTS

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Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21° C. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	ns
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	ns
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	*
945	α-fenchene	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	*
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	ns
990	74,87,43,115	0.5	0.3	0.4	0.3	0.4	0.3	0.4	ns
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	ns
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	ns
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	ns
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	*
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	ns
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	ns
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	ns
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	ns
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	ns
1100	n-nonanal	t	t	0.2	t	0.2	t	t	ns
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	nt
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	*
1186	α-terpineol	t	t	t	t	t	t	t	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	ns
1223	citronellol	0.2	t	t	t	0.2	0.2	t	ns
1261	152,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	ns
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	**
1285	safrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	**

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	F sig
1417	(E)-caryophyllene	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	ns
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	0.2	t	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	ns
1500	α-muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	ns
1513	γ-cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	*
1522	δ-cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	*
1539	α-copaen-11-ol	t	0.3	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	*
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	ns
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	ns
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	*
1630	γ-eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	ns
1638	epi-α-cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	ns
1638	epi-α-muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	ns
1649	β-eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	ns
1652	α-eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	ns
1652	α-cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	ns
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	ns
1688	shyobunol	t	t	t	t	0.2	0.2	t	ns
1746	8-α-11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	ns
1761	iso to 8-α-acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	ns
1792	8-α-acetoxy-elemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	ns
2087	abietadiene	t	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	*
2312	abieta-7,13-dien-3-one	t	t	t	t	t	t	t	nt

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of the leaf oils of *J. virginiana* under extreme drying conditions: 24hrs @ 30º C, 60º C,100º C. * = significant, ** = highly significant, ns = not significant, nt =not tested. Any values that have the same letter (a, b, c, d) following it are not significantly different by the SNK multiple range test (P=0.05).

AI	compound	fresh	30º C	60º C	100º C	F sig.
	percent yield	0.54a	0.50b	0.42c	0.36a	**
924	α-thujene	0.5a	0.5a	0.6a	1.1b	**
932	α-pinene	0.7	0.8	1.0	0.7	**
945	α-fenchene	t	t	0.1	t	nt
969	sabinene	19.6a	21.5a	20.2a	13.9b	**
974	β-pinene	0.3	0.1	0.1	t	nt
988	myrcene	1.3a	1.2a	1.1a	0.3b	**
990	74,87,43,115	0.5a	0.4a	0.3a	0.3a	**
1008	3-carene	0.5a	0.7b	2.0c	0.6a	**
1014	α-terpinene	0.5a	0.4ab	0.3b	0.5ab	ns
1024	limonene	15.0a	15.0a	15.0a	13.3b	*
1025	β-phellandrene	10.8a	10.5a	10.8a	8.8b	*
1054	γ-terpinene	0.7a	0.6a	0.5a	0.9b	**
1065	cis-sabinene hydrate	0.6a	0.5b	0.4b	0.4b	**
1086	terpinolene	0.8a	0.8a	1.0b	0.8a	*
1096	trans-sabinene hydrate	0.3	0.3	0.2	0.2	nt
1097	linalool	0.6	0.4	0.2	0.2	nt
1100	n-nonanal	t	t	t	t	nt
1118	cis-p-menth-2-en-1-ol	0.2	0.1	t	t	nt
1136	trans- p-menth-2-en-1-ol	0.1	t	t	t	nt
1148	citronellal	0.1	t	t	t	nt
1174	terpinen-4-ol	1.7a	1.1b	0.7b	1.0b	**
1186	α-terpineol	t	t	t	t	nt
1195	methyl chavicol	0.1	t	t	0.1	nt
1223	citronellol	t	t	t	0.1	nt
1261	152,123,81,77, aromatic	0.3	0.3	0.3	0.4	nt
1274	pregeijerene B	8.3a	9.3b	8.4a	4.0c	**
1285	safrole	9.0a	8.2a	9.0a	10.7b	**
1322	methyl geranate	0.1	t	0.1	0.1	nt
1350	citronellyl acetate	t	t	0.1	t	nt
1379	geranyl acetate	t	t	t	t	nt
1403	methyl eugenol	1.7a	1.8a	2.1b	2.5c	**
1417	(E)-caryophyllene	t	t	0.7	0.2	nt
1447	43,105,149,178, aromatic	0.2	0.3	0.3	0.2	nt

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AI	compound	fresh	30° C	60° C	100° C	F sig.
1465	cis-muurola-4(14),5-diene	t	t	0.1	t	nt
1491	epi-cubebol	t	0.3	0.2	0.2	nt
1500	α-muurolene	t	0.3	0.2	0.3	nt
1513	γ-cadinene	0.3a	0.5b	0.6bc	0.6bc	**
1522	δ-cadinene	0.6a	1.1c	1.0b	1.1c	**
1539	α-copaen-11-ol	t	t	0.2	0.1	nt
1548	elemol	5.1a	4.7a	4.7a	16.0c	**
1555	elemicin	0.6a	0.6a	0.5ab	0.5b	*
1565	(3Z)-hexenyl benzoate	0.3	0.1	t	t	nt
1574	germacrene-D-4-ol	2.6a	2.8a	3.4b	1.5c	**
1630	γ-eudesmol	0.4	0.4	0.3	t	nt
1638	epi-α-cadinol	0.6	0.7	0.5	0.7	ns
1638	epi-α-muurolol	0.6	0.7	0.5	0.7	ns
1649	β-eudesmol	0.5	0.6	0.4	0.4	ns
1652	α-eudesmol	0.6	0.9	0.6	0.6	ns
1652	α-cadinol	1.2	1.0	0.7	0.7	ns
1670	bulnesol	0.5a	0.4b	0.3c	-d	**
1688	shyobunol	0.2	t	t	t	ns
1746	8-α-11-elemodiol	t	0.1	0.1	0.3	ns
1761	iso to 8-α-acetoxyelemol	0.2	0.1	0.1	0.3	ns
1792	8-α-acetoxyelemol	8.2ac	7.0bc	4.9b	10.5a	*
1933	cyclohexadecanolide	- a	- a	t a	0.5b	**
2054	41,81,137,270, diterpene	0.1a	0.1a	0.1a	- b	**
2087	abietadiene	t a	t a	t a	0.1b	**
2108	71,43,57,123, unknown	t a	t a	0.3b	0.2b	**
2142	41,69,255,298,unknown	t a	t a	0.3b	0.3b	**
2188	E-phytol acetate	t	t	0.1	0.1	ns
2298	4-epi-abietal	0.4	0.4	0.5	0.6	ns
2312	abieta-7,13-dien-3-one	0.1a	0.1a	t b	t b	**
2363	abienol isomer	t a	t a	0.1b	0.1b	**

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 3. Comparison of the composition of leaf oils obtained from
fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21° C for 2
weeks. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non
significant, nt = not tested.

Al	Compound	Fresh	Dried	F sig
	percent yield	1.49	1.10	**
921	tricyclene	0.3	0.2	*
924	α-thujene	1.0	0.8	ns
932	α-pinene	1.5	1.0	ns
946	camphene	0.4	0.3	ns
969	sabinene	27.5	27.1	ns
974	β-pinene	t	t	nt
988	myrcene	2.8	2.9	ns
1002	α-phellandrene	t	t	nt
1008	δ-3-carene	t	t	nt
1014	α-terpinene	2.6	2.5	ns
1020	p-cymene	0.2	0.2	ns
1024	limonene	3.1	3.3	*
1054	γ-terpinene	4.1	3.9	ns
1065	cis-sabinene hydrate	1.6	1.5	ns
1086	terpinolene	1.7	1.4	ns
1098	trans-sabinene hydrate	1.8	1.5	ns
1118	cis-p-menth-2-en-1-ol	0.7	0.7	ns
1141	camphor	22.7	26.0	*
1145	camphene hydrate	0.9	0.9	ns
1148	citronellal	1.1	1.2	ns
1165	borneol	1.4	1.9	**
1174	terpinen-4-ol	10.1	7.5	**
1186	α-terpineol	0.5	0.4	ns
1195	cis-piperitol	0.2	0.8	ns
1207	trans-piperitol	0.3	0.3	ns
1219	coahuilensol, me-ether	t	t	nt
1223	citronellol	3.7	4.0	ns
1284	bornyl acetate	3.6	2.5	*
1298	carvacrol	t	t	nt
1374	α-copaene	t	t	nt
1548	elemol	2.9	3.0	ns
1559	germacrene B	0.2	0.1	ns
1627	1-epi-cubenol	0.1	0.1	ns
1630	γ-eudesmol	0.5	0.5	ns

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AI	Compound	Fresh	Dried	F / sig
1649	β-eudesmol	0.4	0.6	*
1652	α-eudesmol	0.7	0.6	ns
1670	bulnesol	0.3	0.2	ns
1987	manoyl oxide	0.2	0.1	nt
2055	abietatriene	0.1	0.1	nt
2087	abietadiene	0.2	0.1	nt
2???	abietal	0.1	0.1	nt
2312	abieta-7,13-dien-3-one	0.2	0.1	nt

AI = Arithmetic Index on DB-5 column. *Tentatively identified.
Compositional values less than 0.1% are denoted as traces (t).
Unidentified components less than 0.5% are not reported.

A SUMMARY OF LECTOTYPES ASSOCIATED WITH
ALOYSIA PALAU (VERBENACEAE)

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ABSTRACT

A summary of lectotypes associated with the genus *Aloysia* Palau is presented along with notes about each. Two were previously designated by other workers (*Aloysia citrodora* Palau and *Verbena triphylla* L'Her.), while 11 are designated here for the first time: *Aloysia chamaedryfolia* Cham., *Aloysia dusenii* Moldenke, *Aloysia polygalifolia* Cham., *Aloysia urticoides* Cham., *Lippia fiebrigii* Hayek, *Lippia peruviana* Turcz., *Lippia polystachya* Griseb., *Lippia montana* Brandegee, *Lippia scorodonioides var. hypoleuca* Briq., *Lippia virgata var. platyphylla* Briq., and *Lippia wrightii var. macrostachya* Torr. Lectotypification is needed in many cases due to the destruction of material in the Berlin herbarium (B) in 1943. *Phytologia 92(2): 199-205 (August 2, 2010).*

KEY WORDS: *Aloysia, Lippia, Verbena*, Verbenaceae, lectotype

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In the course of conducting taxonomic investigations into the genus *Aloysia* Palau, the need to designate lectotypes for certain associated names became apparent. A more readily accessible forum was deemed preferable to a traditional monograph for ease of citation and acquisition. Eleven lectotypes are designated here for the first time and two, previously designated by other authors, are included for the sake of completeness. The summary below includes known lectotypes, nomenclatural synonyms, and brief notes. In many cases lectotypification is needed due to the destruction of specimens at the Berlin herbarium (B) during World War Two; these are handled in the first section. The second section deals with other cases. It is the sole

aim of the author to address nomenclatural issues and no taxonomic determination is implied.

Types destroyed in Berlin in 1943

World War Two led to the destruction of much material in the herbarium of the Botanical Garden and Botanical Museum in Berlin (B), including many types. Hiepko (1987) details this history and reports the Verbenaceae types were not among the specimens that escaped destruction. Adelbert von Chamisso described several of the species of *Aloysia* here discussed and worked at B from 1819 until shortly before his death in 1838 (Hiepko 1987). While he did not designate single specimens as holotypes, like many authors of the time, it is a commonly accepted practice to designate the specimen housed at the worker's herbarium as such. This type material would have been at B and subsequently destroyed (Hiepko 1987). Thus, following sections 9.9 and 9.10 of the Code (McNeill *et al.* 2006), lectotypification is necessary for Chamisso's species, and for several others based on types destroyed at Berlin, as follows:

Aloysia chamaedryfolia Cham., Linnaea 7: 234. 1832. TYPE: BRAZIL. "Brasilia," without date, *F. Sellow s.n.* (LECTOTYPE [designated here]: W!; ISOLECTOTYPES: G-2!, W!).
≡*Lippia chamaedrifolia* (Cham.) Steud., Nomencl. Bot. ed. 2, 2: 54. 1841.

Photographs of the destroyed type specimen from B are extant (F!, GH!, MO!, NY!) and the lectotype is selected from among the known isotypes. One of the two specimens housed at W is here designated as the lectotype based on its completeness and quality of preservation.

Aloysia dusenii Moldenke, Phytologia 1: 440. 1940. TYPE: BRAZIL. PARANÁ: shrubby campo at Tamandré, 4 Oct 1914, *P. Dusén 1050a* (LECTOTYPE [designated here]: S!; TYPE FRAGMENT: NY!).

Photographs of the destroyed type specimen from B are extant (LL!, NY!). The only isotype known is housed at S and is here designated as the lectotype.

Aloysia polygalifolia Cham., Linnaea 7: 236. 1832. TYPE: BRAZIL. Without date or location, *F. Sellow s.n.* (LECTOTYPE [designated here]: G!).

Photographs of the destroyed type specimen from B are extant (F!, GH!, MO!, NY-2!). The only isotype known is housed at G and is here designated as the lectotype.

Aloysia urticoides Cham., Linnaea 7: 238. 1832. TYPE: BRAZIL. Without specific locality, without date, *F. Sellow s.n.* (LECTOTYPE [designated here]: G!; ISOLECTOTYPES: G!, GH!, NY!, US!).
≡*Lippia urticoides* (Cham.) Steud., Nomencl. Bot. ed. 2, 2: 54. 1841.

This case parallels the previous and a lectotype is selected from among the known isotypes. One of the two specimens housed at G is here designated as the lectotype based on its completeness and quality of preservation.

Lippia fiebrigii Hayek, Bot. Jahrb. Syst. 42: 165. 1908. TYPE: BOLIVIA. TARIJA: Cercado Tarija, 4 Feb 1904, *K. Fiebrig 3036* (LECTOTYPE [designated here]: US!; ISOLECTOTYPES: BM!, F!, G-2!, GH-2!, GOET, IBI, S-2!, W!; TYPE FRAGMENT: F!, NY!; PHOTO OF ISOLECTOTYPE [IBI]: NY!).
≡*Aloysia fiebrigii* (Hayek) Moldenke, Rev. Sudamer. Bot. 4: 15. 1937.

Hayek referenced two collections in the protologue for *Lippia fiebrigii* Hayek, *Fiebrig 3036* and *Weberbauer 4910*. Moldenke, upon making the combination *Aloysia fiebrigii* (Hayek) Moldenke, cited the Fiebrig collection housed at B as the type, and that specimen has been destroyed. Photographs of this specimen are extant (F!, LL!, NY!) and a lectotype is selected from among the known isotypes. The specimen housed at US is here designated as the lectotype for its completeness and quality of preservation.

Lippia peruviana Turcz., Bull. Soc. Nat. Mosc. 36, 2: 200. 1863. TYPE: PERU. Punochuca, without date, *Mathews s.n.* (LECTOTYPE [designated here]: BM!; ISOLECTOTYPE: BR; PHOTO OF ISOLECTOTYPE [BR]: NY!).
≡*Aloysia peruviana* (Turcz.) Moldenke, Rev. Sudamer. Bot. 4: 15. 1937.

The holotype of *Lippia peruviana* Turcz. would have been housed at B and thus destroyed. A lectotype is here designated from among the known isotypes. The specimen housed at BM is here designated as the lectotype for its completeness and quality of preservation.

Lippia polystachya Griseb., Abh. Königl. Ges. Wiss. Göttingen 19: 242. 1874. TYPE: ARGENTINA. CORDOBA: prope Las Mollas ditionis Las Peñas, loco unico obvia, Jan 1871, *P. Lorentz 130* (LECTOTYPE [designated here]: G!; ISOLECTOTYPES: US!, VT).
≡*Aloysia polystachya* (Griseb.) Moldenke, Lilloa 5: 380. 1940.

The holotype of *Lippia polystachya* Griseb. would have been housed at B and thus destroyed. Photographs of this specimen are extant (F!, GH!, MO!, NY-2!) and a lectotype is selected from among the known isotypes. The specimen housed at G is here designated as the lectotype for its completeness and quality of preservation.

Other lectotypes

Aloysia citrodora Palau, Partes Prac. Bot. 1: 768. 1784. TYPE: Un-numbered illustration by B. Salvador y Carmona in appendix of Parte Práctica de la Botánica de Linnaeus. 1784. (LECTOTYPE: MA [designated by Armada and Barra 1992]).
≡*Verbena citrodora* (Palau) Cav., Descript. 68. 1802.

The lectotype for *Aloysia citrodora* Palau was designated by Armada & Barra (1992) as the illustration by B. Salvador y Carmona published in the appendix of Palau's protologue. The use of an illustration, while not common practice, is satisfactory in this case given the uniqueness of the species in question, the exceptional quality of the illustration, and the lack of material annotated by the author. The origin of the cultivated material which served as the basis for the illustration is unknown. One potential specimen exists ("Ortega scripsit", without location, collector, or date [S!], photographs [F!, NY!], photocopy [LL!]) which may have served as type material; however, there is no evidence of this specimen having been examined by Palau.

Lippia montana Brandegee, Proc. Calif. Acad. Sci. ser. 2, 3: 163. 1891.
TYPE: San Bartolomé, Oct 10 1889, *T. S. Brandegee s.n.*
(LECTOTYPE [here designated]: UC!; ISOLECTOTYPE: US!).

Brandegee cited three of his own collections in the protologue for *Lippia montana* Brandegee; Sierra de la Laguna (UC!), San José del Cabo (NY!, UC!), and San Bartolomé (UC!, US!). The Brandegee collection from San Bartolomé housed at UC is here designated as the lectotype for its completeness, quality of preservation, and its location in Brandegee's former working herbarium.

Lippia scorodonioides **var. *hypoleuca*** Briq., Bull. Herb. Boiss. 4: 338.
1896. TYPE: PERU. Without date, *Dombey 259* (LECTOTYPE [here designated]: G!; PHOTO OF LECTOTYPE: F!, MO!, NY!).
≡*Aloysia scorodonioides var. hypoleuca* (Briq.) Moldenke, Phytologia 36: 437. 1977.

Briquet cites two collections in the protologue for *Lippia scorodonioides* var. *hypoleuca*, *Dombey 259* and *Mandon 522*. He goes on to state "specimen Mandonnianum jam ad var. sequentem transit" indicating that he probably considered the Dombey collection to be more typical of the taxon. Therefore, the Dombey specimen housed at G is here designated as the lectotype based on the quality of preservation and it's location in Briquet's former working herbarium.

Lippia virgata **var. *platyphylla*** Briq., Ann. Conserv. Jard. Bot. Genéve
7-8: 304. 1904. TYPE: PARAGUAY. Paraguari, Mar 1881, *B. Balansa 3116* (LECTOTYPE [designated here]: F!; ISOLECTOTYPE: GH!).
≡*Aloysia virgata var. platyphylla* (Briq.) Moldenke, Phytologia 2: 408. 1948.

Briquet cites two collections in the protologue for *Lippia virgata* var. *platyphylla*, *Balansa 1016 pro parte* and *Balansa 3116*. He goes on to cite the remainder of *Balansa 1016* as the type for *L. virgata* var. *elliptica*. Since there is no material labeled *Balansa 1016* housed at Briquet's former working herbarium (G), and to avoid confusion amongst these collections, *Balansa 3116* is chosen as the type for *L. virgata* var. *platyphylla*. The specimen housed at F is here designated as the lectotype for its completeness and quality of preservation.

Lippia wrightii* var. *macrostachya Torr., in Emory, Rep. U.S. & Mex.
    Bound. Surv. 2: 126-127. 1859. TYPE: MÉXICO. NUEVO
    LEON: W of Cerralbo, 28 May 1847, *J. Gregg 822* (LECTOTYPE
    [here designated]: NY!; ISOLECTOTYPE: MO!).
≡*Aloysia macrostachya* (Torr.) Moldenke, Phytologia 1: 95. 1934.

    Torrey cites two collections in his protologue for *Lippia wrightii*
var. *macrostachya*, *Gregg 822* and *Schott s.n.* The Gregg collection is
chosen over that of Schott because it is more complete and there is a
duplicate of *Gregg 822* housed at MO. The specimen housed at NY
does not actually have the collection number (822) on the label, but it
bears Torrey's autograph, matches the label data from the numbered
specimen at MO, and is deposited in Torrey's former working
herbarium, leaving no doubt that this is the specimen cited in the
protologue. Therefore, the specimen housed at NY is here designated as
the lectotype.

Verbena triphylla L'Her., Stirp. Nov. 1: 21, pl. 11. 1785. TYPE:
    FRANCE. ILE DE FRANCE: cultivated plant in the Jardin de
    Plantes, Paris, without date, *C. L. L'Heritier s.n.* (LECTOTYPE: P
    [designated by Moldenke & Moldenke 1983]).
≡*Aloysia triphylla* (L'Her.) Britton, Sci. Surv. Porto Rico and Virgin
    Islands 6: 140. 1925.
≡*Lippia triphylla* (L'Her.) Kuntze, Rev. Gen. Pl. 3: 253. 1898.

    Moldenke and Moldenke (1983) designated the lectotype for
Verbena triphylla L'Her. as *L'Heritier s.n.* (P) and report the material
was chosen "from seed sent by Commerson from Montevideo,
Uruguay." Indeed, in his protologue L'Heritier reports the "habitat in
Chile. Dombey. in Bonaria: Monti-Video. Philib. Commerson" which
would indicate either Dombey or Commerson collections from Chile or
Uruguay as potential sources. However, L'Heritier goes on to state
"viviam necnon semina bunigne communicavit praedictus Ortega"
indicating the seeds were probably sent by Ortega. Indeed, the lectotype
housed at P is from cultivated material and does not represent a wild
collection. No specimens attributable to either Dombey or Commerson
have been uncovered which may serve as complementary material.

ACKNOWLEDGEMENTS

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THE VASCULAR FLORA OF A RIVER BOTTOM IN EAST CENTRAL MISSISSIPPI

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Author's Note: In 1976, this paper, under the title "The Vascular Flora of the Chitlin' Corners Area of Lowndes County, Mississippi," was submitted to *Castanea* for consideration for publication. The paper was rejected, not for deficiencies, but because the journal, in an effort to improve the quality of its content, was no longer publishing floristic checklists. A copy of the manuscript was recently discovered and appears here in basically an unaltered state, other than the nomenclature being updated from 1976 standards to that of USDA, NRCS (2010) and the addition of collection numbers of each species. It is believed that this may be the only record of the flora of the lower portion of the Tombigbee River area of Mississippi prior to the creation of the Tennessee-Tombigbee Waterway and the subsequent raising of the water level. *Phytologia 92(2): 206-229 (August 2, 2010)*.

ABSTRACT

The study area, known locally as "Chitlin' Corners," is located on the west bank of the Tombigbee River in Lowndes County in east central Mississippi. Five hundred and ninety-one species are recorded for this area, prior to the construction the Tennessee-Tombigbee Waterway. Among the more unusual plants for east central Mississippi are *Astragalus distortus, Phacelia dubia, Carex grayi, Opuntia pusilla,* and *Tetragonotheca helianthoides*.

KEY WORDS: Mississippi, Lowndes County, Tombigbee River, Tennessee-Tombigbee Waterway, floristics.

The study area, locally known as "Chitlin' Corners," is located on the west bank of the Tombigbee River in the southeast corner of Lowndes County in east central Mississippi. The area includes parts of sections 19, 20, and 28 of Township 20S, Range 19E. Prominent features include the Tombigbee River with its bluffs, mesic woodlands, drainage and swampy areas, oxbow lakes, streams, and oil fields. At the time of the study, there were only a few recreational camps in the area. The major man-made feature was the Southern Natural Gas Pipeline, which crosses the river at this point.

This work comprises the most extensive study of the vascular flora of the Tombigbee River in Mississippi River prior to the now in-progress construction of the Tennessee-Tombigbee Waterway. Since it is not known exactly what changes will accompany the construction and use of the waterway, it is desirable to place on permanent and readily accessible record the results of the study. One previous botanical study was made in the area. Watson and Brown (1967) investigated the arborescent flora, reporting 96 species of trees occurring naturally in the area. The species recorded by Watson and Brown are herein included, with permission, and are annotated by an asterisk.

Geology and Topography

Relief in the area is slight, varying between 42-49 meters. Most of the area appears level. The underlying geologic unit is the Mooreville Chalk (Selma Group) of Upper Cretaceous age (Mississippi Geological Society, 1945). Soils are alluvial and consist of fine sands and sandy loams (Watson and Brown, 1967). Most of the area is subject to periodic inundation, especially the low-lying areas and drainages. The major geologic features of interest were the bluffs which drop abruptly about six meters to the river's edge. In these bluffs, exposed chalk (bedrock) is common. Other features of interest are the oxbow lakes.

Plant Community Types

Although relief is slight, marked variation in communities is easily recognized. The following is a listing of the major communities.

Wooded bluffs: Wooded bluffs have steep slopes with flat, level tops. Seepage areas are common. Plants include *Fagus grandifolia, Tilia* sp., *Liriodendron tulipifera, Mitchella repens,* and *Polystichum acrostichoides*. The bluff tops are drier and include *Pteridium aquilinum* var. *pseudocaudatum, Aureolaria flava, Quercus austrina, Crataegus* sp., *Vaccinium* sp., and *Pinus* sp. *Ludwigia decurrens, Heliotropium indicum, Laportea canadensis,* and *Sagittaria latifolia* are common where the seepage areas meet the river.

Open bluffs: These bluffs are practically void of arborescent flora and appear to be scoured by the river and subject to periodic landslides. The sites are dry with chalk exposed in places. Common species include *Ampelopsis arborea, Ipomoea* sp., *Xanthium strumarium, Eupatorium serotinum,* and *Oenothera biennis.*

Shrub areas (old fields): The shrub area provides the most interesting area for study. Typically, the area is level, has sandy soil, is normally dry, and is not often flooded. Characteristic species include *Vaccinium* sp., *Crataegus* sp., *Celtis tenuifolia* var. *georgiana, Prunus* sp., *Opuntia pusilla,* and *Yucca filamentosa.* Herbaceous plants include *Rumex hastatulus, Gaillardia aestivalis, Solidago discoides, Conyza canadensis, Pseudognaphalium obtusifolium,* and *Cnidoscolus stimulosus.*

Drainages, lowlands, and wet areas: The areas are periodically inundated and normally moist during most of the year. The topography varies from low-swampy to small ridges that border the low places. Such trees as *Taxodium distichum, Carya aquatica,* and *Nyssa aquatica* are common. Other plants include *Smilax* sp., *Berchemia scandens, Arisaema dracontium,* and *Saururus cernuus.* On the higher areas that border the low areas, plants such as *Chasmanthium latifolium, Carex* sp., *Toxicodendron radicans,* and *Parthenocissus quinquefolia* are common. The more open areas, including some that border on the river, are characterized by *Polygonum lapathifolium, Hibiscus laevis, Polygonum pensylvanicum, Xanthium strumarium,* and *Phyla lanceolata.*

Woodlands: Woodlands are found on higher sites. Some slope gently to the river or other low areas. Dominant plants include *Quercus alba,*

Quercus falcata, Carya alba, and *Prunus serotina.* Other plants include *Euonymus americana, Lindera benzoin, Galium* sp., *Zizia aurea, Thaspium trifoliata, Viola affinis, Sanicula canadensis,* and *Chasmanthium sessilifolium.*

Floristics

The Chitlin' Corners area has a rich vascular flora for east central Mississippi. Some of the more unusual plants found along with comments are listed below.

Astragalus distortus and *Phacelia dubia* are of unusual occurrence in Mississippi, both being very common in open areas during March and April. *Carex grayi* is normally found much further north, this probably being the southernmost extent of its range. *Opuntia pusilla* and *Tetragonotheca helianthoides* are typical of the longleaf pine section of Mississippi. Other interesting plants include *Heliopsis helianthoides, Stylisma humistrata, Stylisma patens, Callirhoe triangulata, Sida elliottii, Menispermum canadense,* and *Melanthera nivea.* Watson and Brown (1967) recorded the following woody species to be of unusual occurrence in east central Mississippi: *Carya pallida, Quercus margaretta, Quercus incana, Quercus laurifolia, Celtis tenuifolia, Sideroxylon lycioides, Quercus texana, Prunus caroliniana,* and *Ilex vomitoria.*

Following is a list of the 591 species of plants known to occur in the Chitlin' Corners area. Nomenclature generally follows that of Radford et al. (1968). Species not native to Mississippi are indicated with a section symbol (§). [Names from Watson and Brown (1967) are noted with an asterisk(*). Collection numbers (all WCH), which follow each species name, were not in the original manuscript.]

The study began April, 1972 and was completed in October 1973. Approximately 30 collecting trips were made. Presently voucher specimens are located at the Northwestern State University Herbarium (NATC), but will eventually be deposited in the personal herbarium of Sidney McDaniel of the Institute for Botanical Exploration, Mississippi State, Mississippi.

Pteridophyta
Filicopsida

Aspleniaceae
Asplenium platyneuron (L.) B.S.P. 1048

Azollaceae
> *Azolla caroliniana* Willd. 2045

Blechnaceae
> *Woodwardia areolata* (L.) T. Moore 1186

Dennstaedtiaceae
> *Pteridium aquilinum* (L.) Kuhn var. *pseudocaudatum* (Clute)
>> Heller 1052

Dryopteridaceae
> *Onoclea sensibilis* L. 1253
> *Polystichum acrostichoides* (Michx.) Schott 902

Ophioglossaceae
> *Botrychium dissectum* Spreng. 1571
> *Botrychium virginianum* (L.) Sw. 948, 1072, 1118

Osmundaceae
> *Osmunda regalis* L. 1856

Polypodiaceae
> *Pleopeltis polypodioides* ssp. *polypodioides* (L.) Andrews &
>> Windham 901

Coniferophyta
Pinopsida

Cupressaceae
> **Juniperus virginiana* L.

Pinaceae
> **Pinus echinata* P. Mill.
> **Pinus taeda* L.

Taxodiaceae
> **Taxodium distichum* (L.) Rich.

Magnoliophyta
Liliopsida

Agavaceae
> *Yucca filamentosa* L. 1175

Alistmataceae
> *Sagittaria latifolia* Willd. 1401

Araceae
> *Arisaema dracontium* (L.) Schott 943

Arecaceae
> *Sabal minor* (Jacq.) Pers. 1737

Commelinaceae

       Commelina diffusa Burm. f. 1139

       Commelina erecta L. 1165

       Tradescantia ohiensis Raf. 1021, 1108

Cyperaceae

§   *Bulbostylis barbata* (Rottb.) C.B. Clarke 1512

       Carex abscondita Mackenzie 1846

       Carex amphibola Steud. 1050

       Carex bushii Mackenzie 1020

       Carex caroliniana Schwein. 964A

       Carex cherokeensis Schwein. 1722

       Carex crebriflora Wieg. 1727

       Carex digitalis Willd. 925

       Carex grayi Carey 936

       Carex intumescens 1724

       Carex joorii Bailey 1882

       Carex lupulina Muhl. ex. Willd. 1718

       Carex muehlenbergii var. *muehlenbergii* Schkuhr ex Willd.
           1043

       Carex retroflexa Muhl. ex. Willd. 959

       Carex rosea Schkuhr ex Willd. *s.n.*

       Carex striatula Michx. 912

       Carex triangularis Boeckl. 1732

       Cyperus croceus Vahl *s.n.*

       Cyperus echinatus (L.) Wood *s.n.*

       Cyperus erythrorhizos Muhl. 1420, 1509

       Cyperus pseudovegetus Steud. *s.n.*

       Cyperus retrorsus Chapman 1539

§   *Cyperus rotundus* L. *s.n.*

       Cyperus strigosus L. 1982

       Eleocharis microcarpa Torr. 1412

       Eleocharis obtusa (Willd.) J.A. Schultes 997

       Kyllinga brevifolia Rottb. 1970A

       Scirpus cyperinus (L.) Kunth 1822

       Scleria triglomerata Michx. 1736

Dioscoreaceae

       Dioscorea villosa L. 1117

Iridaceae

       Iris virginica L. 1865

Sisyrinchium albidum Raf. 906A, 961A, 1064
Sisyrinchium angustifolium P. Mill. 961
Sisyrinchium atlanticum Bickn. 963

Juncaceae

Juncus coriaceus Mackenzie 1106
Juncus dichotomus Ell. 1107
Juncus marginatus Rostk. 1010
Juncus repens Michx. 1863
Juncus tenuis Willd. 1101, 1202
Luzula echinata (Small) F.J. Herm. 935, 2050

Lemnaceae

Lemna perpusilla Torr. 982
Spirodela polyrrhiza (L.) Schleid. 1859A

Liliaceae

Allium canadense var. *canadense* L. 962, 1030
Allium canadense var. *mobilense* L. (Regel) Ownbey
Chamaelirium luteum (L.) Gray 1032
Hypoxis hirsuta (L.) 1023
Lilium michauxii Poir. 1322, 1870
Maianthemum racemosum ssp. *racemosum* (L.) Link 1099
Manfreda virginica (L.) Salisb. ex Rose 1365, 1560
Polygonatum biflorum (Walt.) Ell. 940, 1049
Trillium cuneatum Raf. 910

Poaceae

Agrostis perennans (Walt.) Tuckerm. 1655
Alopecurus carolinianus Walt. 1022
Aristida basiramea Engelm. ex Vasey 1530
Aristida dichotomas var. *curtissii* Gray ex S. Wats. & Coult.
　　1629
Aristida lanosa Muhl. ex Ell. 1528
Arundinaria gigantea (Walt.) Muhl. 1033, 1278
§　*Avena sativa* L. 1002
Cenchrus longispinus (Hack.) Fern. 1220, 1435
Cenchrus spinifex (Cav.) 1521
Chasmanthium latifolium (Michx.) Yates. 1197, 1212, 1256
Chasmanthium sessiliflorum (Poir.) Yates. 1267, 1297
§　*Cynodon dactylon* (L.) Pers. 1180
Danthonia sericea Nutt. 1720
Dichanthelium boscii (Poir.) Gould & C.A. Clark 1137

Dichanthelium commutatum (J.A. Schultes) Gould 999, 1056, 1097, 1128, 1131, 1618, 1486

Dichanthelium consanguineum (Kunth) Gould & C.A. Clark 1102, 1122

Dichanthelium dichotomum var. *dichotomum* (L.) Gould 1042, 1103, 1385

Dichanthelium laxiflorum (Lam.) Gould 1012

Dichanthelium oligosanthes var. *oligosanthes* (J.A. Schultes) Gould 991, 1056A

Dichanthelium scabriusculum (Ell.) Gould & C.A. Clark 1104

Dichanthelium scoparium (Lam.) Gould 1029

Dichanthelium sphaerocarpon var. *sphaerocarpon* (Ell.) Gould 1098, 1129, 1130

Digitaria cognata var. *cognata* (J.A. Schultes) Pilger 1628

Digitaria sanguinalis (L.) Scop. 1199, 1344, 1423, 1445, 1515, 1602

Digitaria villosa (Walt.) Pers. 1409, 1516

Danthonia sericea Nutt. 1720

Echinochloa colonum (L.) Link. 1847

§   *Echinochloa crus-galli* (L.) Beauv. 1369, 1458

§   *Eleusine indica* (L.) Gaertn. 1497, 1554

Elymus virginicus L. 1153, 1500

§   *Eragrostis cilianensis* (All.) Vign. ex Janchen 1201

Eragrostis hirsuta (Michx.) Nees 1414, 1483

Eragrostis hypnoides (Lam.) BSP. 1421

§   *Eragrostis japonica* (Thunb.) Trin. 1327

Eragrostis pilosa (L.) Beauv. 1424

Eragrostis spectabilis (Purch) Steud. 1303

Gymnopogon ambiguus (Michx.) BSP. 1608

Hordeum pusillum Nutt.

Leersia oryzoides (L.) Sw. 1529

Leersia virginica Willd. 1501

Lolium perenne ssp. *multiflorum* (Lam.) Husnot 1210

§   *Lolium pratense* (Huds.) S.J. Darbyshire 1105

Melica mutica Walt. 927

Panicum anceps Michx. 1619, 1666, 1672

Panicum dichotomiflorum Michx. 1422, 1490

Panicum verrucosum Muhl. 1418

Panicum virgatum L. 1310, 1368, 1496

Paspalum boscianum Flueggé 1590
Paspalum floridanum Michx. 1366, 1448
Paspalum fluitans (Ell.) Kunth 1450
Paspalum setaceum Michx. 1441
§ *Paspalum urvillei* Steud. 1213, 1390
Piptochaetium avenaceum (L.) Parodi 929, 978
§ *Poa annua* L. 913
Poa autumnalis Muhl. ex Ell. 1019, 1096
Saccharum alopecuroidum (L.) Nutt. 1413
Saccharum brevibarbe var. *contortum* (Ell.) R. Webster 1517, 1526
Sacciolepis striata (L.) Nash 1674
Schizachyrium scoparium var. *scoparium* (Michx.) Nash 1477
Setaria parviflora (Poir.) Kerguélen 1138, 1430, 1545, 1658
Sorghum halepense (L.) Pers. 1181
§ *Sphenopholis filiformis* (Chapman) Scribn. 965
Sphenopholis nitida (Biehler) Scribn. 926
Sphenopholis obtusata (Michx.) Scribn. 1100
Sporobolus clandestinus (Biehler) A.S. Hitchc. 1564
Sporobolus junceus (Beauv.) Kunth 1551, 1610
Sporobolus indicus var. *indicus* (L.) R. Br. 1269, 1526
Tridens flavus (L.) A.S. Hitchc. 1482, 1518, 1670
Tridens strictus (Nutt.) Nash 1057, 1392, 1615
Tripsacum dactyloides (L.) L. 1178
§ *Triticum aestivum* L. 1004
Vulpia octoflora var. *octoflora* (Walt.) Rydb.
Smilacaceae
Smilax bona-nox L. 1670
Smilax rotundifolia L. 1451
Smilax smallii Morong 1046, 1685
Smilax tamnoides L. 1024, 1044
                                Magnoliopsida
Acanthaceae
Justicia americana (L.) Vahl 1026, 1154, 1242A, 1329
Ruellia caroliniensis (J.F. Gmel.) Steud. 1239
Ruellia humilis Nutt. 1251, 1538
Ruellia strepens L. 1077, 1182, 1384
Aceraceae
* *Acer negundo* L.

        * *Acer rubrum* var. *trilobum* Torr. & Gray ex. K. Koch
        * *Acer saccharinum* L.
Amaranthaceae
        Amaranthus rudis Sauer 1403
        Froelichia floridana (Nutt.) Moq. 1342
        Iresine rhizomatosa Standl. 1286, 1399, 1449
Anacardiaceae
        Rhus copallina L. 1398
        Rhus glabra L. 1357
        Toxicodendron pubescens P. Mill. 985
        Toxicodendron radicans ssp. *radicans* (L.) Kuntze 1698
Annonaceae
        Asimina parviflora (Michx.) Dunal 1194
        * *Asimina triloba* (L.) Dunal
Apiaceae
        Chaerophyllum tainturieri Hook. 907, 954
        Cicuta maculata L. 1231
        Cryptotaenia canadensis (L.) DC. 1086, 1232, 1238
§   *Daucus carota* L. 1127
        Daucus pusillus Michx. 1088, 1183, 1261
        Eryngium yuccifolium Michx. 1555
        Ptilimnium capillaceum (Michx.) Raf. 1148
        Sanicula canadensis L. 1184
        Spermolepis divaricata (Walt.) Raf. ex Ser. 1040, 1061
        Thaspium trifoliatum (L.) Gray 993, 1276, 1284, 1474, 1649
        Trepocarpus aethusae Nutt. ex DC. 1227, 1294
        Zizia aurea (L.) W.D.J. Koch 932
Apocynaceae (including Asclepidaceae)
        Amsonia tabernaemontana   var. salicifolia (Pursh) Woods.
            921, 979, 1050, 1111
        Asclepias tuberosa L. 1188
        Asclepias variegata L. 1055, 1542
        Asclepias verticillata L. 1353
        Matelea gonocarpa (Walter) Shinners 1193, 1510
        Trachelospermum difforme (Walt.) Gray 1880
Aquifoliaceae
        * *Ilex ambigua* (Michx.) Torr.
        * *Ilex decidua* Walt.
        * *Ilex opaca* Ait.

      * *Ilex vomitoria* Ait.
Araliaceae
      **Aralia spinosa* L.
Aristolochiaceae
      Aristolochia tomentosa Sims 1854
Asteraceae
      Acmella oppositifolia var. *repens* (Walt.) R.K. Jansen 1983
      Ageratina altissima var. *altissima* (L.) King & H.E. Robins. 1678
      Ambrosia artemisiifolia L. 1464, 1484, 1520
      Ambrosia trifida L. 1459, 1499
      Antennaria plantaginifolia (L.) Richards. 2058
      Bidens aristosa (Michx.) Britt. 1436, 1595
      Bidens bipinnata L. 1363, 1452, 1491, 1626
      Bidens mitis (Michx.) Sherff 1473, 1556, 1609
      Boltonia diffusa Ell. 1229, 1360, 1386
      Brickellia eupatorioides var. *eupatorioides* (L.) Shinners 1488, 1546
      Chrysopsis mariana (L.) Ell. 1519, 1650, 1652
      Chrysopsis pilosa Nutt. 1301, 1455, 1561, 1673
      Cirsium discolor (Muhl. ex Willd.) Spreng. 1205
      Conoclinium coelestinum (L.) DC. 1203, 1308, 1362
      Conyza canadensis (L.) Cronq. 1274, 1426, 1624, 1643, 1644
      Coreopsis auriculata L. 1206
      Coreopsis basalis (A. Dietr.) S.F. Blake 1060, 1081
      Coreopsis major Walt. 1190, 1373
      Croptilon divaricatum (Nutt.) Raf. 1425, 1514, 1625
      Eclipta prostrata (L.) L. 1283, 1379, 1469
      Elephantopus carolinianus Raeusch. 1354
      Elephantopus tomentosus L. 1295, 1364, 1873
      Erechtites hieracifolia (L.) Raf. ex DC. 1593
      Erigeron philadelphicus L. 996
      Erigeron strigosus Muhl. ex Willd. 1045, 1126, 1540
      Eupatorium capillifolium (Lam.) Small 1574
      Eupatorium serotinum Michx. 1338, 1475, 1489, 1657
      Eurybia hemispherica (Alexander) Nesom 1395, 1645, 1899
      Euthamia tenuifolia var. *tenuifolia* (Pursh) Nutt. 1622
      Fleischmannia incarnata (Walt.) King & H.E. Robins. 1489A, 1599, 1679

Gaillardia aestivalis (Walt.) H. Rock 1058, 1024, 1136, 1280, 1480, 1523

Gamochaeta purpurea (L.) Cabrera 924, 1014

Helenium amarum (Raf.) H. Rock 1204

Helianthus angustifolius L. 1376

Helianthus giganteus L. *s.n.*

Heliopsis helianthoides (L.) Sweet 1059, 1079, 1167

Heterotheca subaxillaris (Lam.) Britt. & Rusby 1258, 1456

Hieracium gronovii L. 1244, 1318, 1378, 1479

Ionactis linariifolius (L.) Greene 1607, 1661

Iva annua L. 1591

Krigia caespitosa (Raf.) Chambers 973

Krigia virginica (L.) Willd. 918A

Lactuca canadensis L. 1299

Lactuca floridana (L.) Gaertn. 1507

Liatris aspera Michx. 1841

Liatris squarrulosa Michx. 1332, 1838

Melanthera nivea (L.) Small 1361, 1367, 1375

Mikania scandens (L.) Willd. 1298, 1407, 1527

Packera anonyma (Wood) W.A. Weber & A. Löve 1713

Packera glabella (Poir.) C. Jeffrey 968, 988

Pluchea camphorata (L.) DC. 1394, 1428, 1659

Prenanthes altissima L. 1647

Pseudognaphalium obtusifolium ssp. *obtusifolium* (L.) Hilliard & B.L. Burtt 1302, 1405, 1481, 1522, 1550

Pyrrhopappus carolinianus (Walt.) DC. 1080, 1277

Rudbeckia hirta L. 1173

Silphium asteriscus var. *laevicaule* (L.) DC. 1271

Silphium radula Nutt. 1828, 1896

Solidago arguta var. *boottii* (Hook.) Palmer & Steyermark

Solidago canadensis L. 1347, 1437

Solidago canadensis var. *scabra* Torr. & Gray 1335, 1537, 1553, 1643

Solidago caesia L. 1406, 1506

Solidago discoidea Ell. 1305, 1320, 1381, 1487, 1513

Solidago odora Ait. 1511, 1664

Solidago ulmifolia Muhl. Ex Willd.

Symphyotrichum concolor (L.) Nesom 1606

Symphyotrichum cordifolium (L.) Nesom 1568, 1572, 1646, 1680

Symphyotrichum divaricatum (Nutt.) Nesom *s.n.*

Symphyotrichum ericoides var. *ericoides* (L.) Nesom 1502

Symphyotrichum lanceolatum var. *lanceolatum* (Willd.) Nesom 1667

Symphyotrichum lateriflorum var. *lateriflorum* (L.) A.&D. Löve 1576

Symphyotrichum patens var. *patens* (Ait.) Nesom 1562, 1605, 1653

Symphyotrichum pilosum var. *pilosum* (Willd.) Nesom *s.n.*

Symphyotrichum racemosum (Ell.) Nesom 1387, 1493

Tetragonotheca helianthoides L. 1119

Verbesina virginica L. 1492

Vernonia gigantea ssp. *gigantea* (Walt.) Trel. 1255, 1346, 1498, 1505

Xanthium strumarium L. 1465

Balsaminaceae

Impatiens capensis Meerb. 1831

Berberidaceae

Podophyllum peltatum L. 1073

Betulaceae

*Betula nigra L.

*Carpinus caroliniana Walt.

Ostrya virginiana (P. Mill) K. Koch 1878

Bignoniaceae

Bignonia capreolata L. 945, 1687

Campsis radicans (L.) Seem. ex Bureau 1191

Boraginaceae

§   *Heliotropium indicum* L. 1323, 1383

Myosotis macrosperma Engelm. 1715

Onosmodium molle ssp. *hispidissimum* (Mackenzie) Boivin 1093

Brassicaceae

§   *Arabidopsis thaliana* (L.) Heynh. 918B, 980, 1012

Arabis laevigata (Muhl. ex Willd.) Poir. 1084, 1704

§   *Brassica rapa* var. *rapa* L. 906

§   *Brassica juncea* (L.) Czern. 1690

§   *Capsella bursa-pastoris* (L.) Medik. 1697

   Cardamine bulbosa (Schreb. ex Muhl.) B.S.P 2046
   Cardamine parviflora L. 914
   Lepidium virginicum L. 965A, 1039
   Rorippa sessiliflora (Nutt.) A.S. Hitchc. 923, 1027, 1087,
    1164, 1840

Buddlejaceae
   Polypremum procumbens L. 1236, 1326

Cactaceae
   Opuntia pusilla (Haw.) Nutt. 1069, 1078

Campanulaceae
   Lobelia cardinalis L. 1876
   Lobelia inflata L. 1897
   Lobelia puberula Michx. 1675
   Triodanis perfoliata var. *perfoliata* (L.) Nieuwl. 995

Caprifoliaceae
§ *Lonicera japonica* Thunb. 1016
   Sambucus nigra ssp. *canadensis* (L.) R. Bolli 1192
   *Viburnum rufidulum Raf.

Caryophyllaceae
§ *Arenaria serpyllifolia* L. 969, 1066, 1095
   Cerastium glomeratum Thuill. 915, 920
   Silene stellata (L.) Ait. f. 1355
   Silene virginica L. 987
§ *Stellaria media* (L.) Vill. 1708

Celastraceae
   Euonymus americanus L. 1116

Chenopodiaceae
   Chenopodium album L. 1218, 1288, 1431, 1476, 1533, 1544,
    1558, 1669
§ *Chenopodium ambrosioides* L. 1447

Cistaceae
   Helianthemum georgianum Chapman 1159, 1630, 1803
   Lechea mucronata Raf. 1222

Clusiaceae
   Hypericum drummondii (Grev. & Hook.) Torr. & Grey 1535
   Hypericum hypericoides (L.) Crantz 1569
   Hypericum lobocarpum Gattinger 1265, 1321, 1548
   Hypericum mutilum L. 1833
   Triadenum tubulosum (Walt.) Gleason 1440

Convolvulaceae

    Calystegia sepium (L.) R. Brown 1196

    Cuscuta gronovii Willd. ex J.A. Schultes 1597

    Dichondra carolinensis Michx. 1094

    Ipomoea lacunosa L. 1151, 1417A

    Ipomoea nil (L.) Roth 1417

    Ipomoea pandurata (L.) G.F.W. Mey. 1315, 1341

    Jacquemontia tamnifolia (L.) Griseb. 1551

    Stylisma humistrata (Walt.) Chapman 1230

    Stylisma patens ssp. *patens* (Desr.) Myint 1162

Cornaceae

    *Cornus florida L.

    *Cornus foemina P. Mill.

Crassulaceae

    Penthorum sedoides L. 1025, 1389, 1655

Cucurbitaceae

    Cayaponia quinqueloba (Raf.) Shinners 1337

    Sicyos angulatus L. 1404, 1478

Ebenaceae

    *Diospyros virginiana L.

Ericaceae

    *Oxydendrum arboreum (L.) DC.

    Rhododendron canescens (Michx.) Sweet 1031

    Vaccinium arboreum Marsh. 960, 1031

    Vaccinium stamineum L. 958

    Vaccinium elliottii Chapman 1712

Euphorbiaceae

    Acalypha gracilens Gray 1532

    Acalypha rhomboidea Raf. *s.n.*

    Chamaesyce maculata (L.) Small 1216, 1220A, 1388, 1408,
        1432, 1676

    Cnidoscolus stimulosus (Michx.) Engelm. & Gray 992, 1134

    Croton capitatus Michx. 1541

    Croton glandulosus L. 1215, 1289, 1434, 1563

    Euphorbia corollata L. 1109, 1157, 1226, 1275, 1433

    Euphorbia dentata Michx. 1330

    Phyllanthus caroliniensis Walt. 1620

    Sebastiania fruticosa (Bartr.) Fern. 1110

    Tragia urens L. 1827

Fabaceae

§   *Albizia julibrissin* Durazz.

      Amorpha fruticosa L. 1132

      Amphicarpa bracteata (L.) Fernald 1312, 1331

      Apios americana Medik. 1370

      Astragalus distortus Torr. & Gray 916, 937, 1699

      Baptisia alba var. *macrophylla* (Larisey) Isely 1734

      Cercis canadensis L.

      Chamaecrista fasciculata var. *fasciculata* (Michx.) Greene 1588

      Chamaecrista nictitans var. *nictitans* (L.) Moench 1377

      Clitoria mariana L. 1113

      Crotalaria sagittalis L. 1161

      Desmodium glabellum (Michx.) DC. 1438

      Desmodium glutinosum (Muhl. ex Willd.) Wood 1279

      Desmodium nuttallii (Schindl.) Schub. 1552

      Desmodium paniculatum (L.) DC. 1512, 1973

      Desmodium pauciflorum (Nutt.) DC. 1158, 1848

      Dioclea multiflora (Torr. & Gray) C. Mohr 1120, 1185

      * *Gleditsia triacanthos* L.

§   *Lespedeza cuneata* (Dum.-Cours.) G. Don 1292

      Lespedeza stuevei Nutt. 1336, 1648

      Mimosa microphylla Dry. 1082, 1358

      Robinia pseudo-acacia L.

      Rhynchosia tomentosa (L.) Hook. & Arn. 1235, 1319, 1825

      Senna marilandica (L.) Link 1870

      Sesbania herbacea (P. Mill) McVaugh 1454

      Strophostyles helvola (L.) Ell. 1219, 1287

      Stylosanthes biflora (L.) B.S.P 1147, 1470

      Tephrosia spicata (Walt.) Torr. & Gray 1163, 1264, 1300

§   *Trifolium dubium* Sibthorp 933

§   *Trifolium lappaceum* L. 1145

§   *Trifolium resupinatum* L. 1705

§   *Vicia sativa* ssp. *nigra* (L.) Ehrh. 917, 942

      Vicia minutiflora F.G. Dietr. 2059,

§   *Vicia villosa* var. *varia* (Host) Corb. 1006

Fagaceae

      Castanea dentata (Marsh.) Borkh.

      Castanea pumila (L.) P. Mill.

        *Fagus grandifolia Ehrh.
        *Quercus alba L.
        *Quercus austrina Small
        * *Quercus coccinea* Meunchh.
        * *Quercus falcata* Michx.
        * *Quercus incana* Bartr.
        * *Quercus laurifolia* Michx.
        * *Quercus lyrata* Walt.
        * *Quercus margaretta*
        * *Quercus michauxii* Nutt.
        * *Quercus muehlenbergii* Engelm.
        * *Quercus nigra* L.
        * *Quercus nuttallii*
        * *Quercus pagoda* (Ell.) Ashe
        * *Quercus phellos* L.
        * *Quercus rubra* L.
        * *Quercus texana* Buckl.
        * *Quercus shumardii* Buckl.
        *Quercus stellata Wangenh.
        * *Quercus velutina* Lam.
Geraniaceae
        Geranium carolinianum L. 998, 1034
Grossulariaceae
        Itea virginica L. 1463
Hamamelidaceae
        * *Hamamelis virginiana* L.
        * *Liquidambar styraciflua* L.
Hippocastanaceae
        * *Aesculus pavia* L.
Hydrangeaceae
        Hydrangea arborescens L. 1155, 1573
Hydrophyllaceae
        Hydrolea ovata Nutt. ex Choisy 1872
        Phacelia dubia (L.) Trel. 938, 966, 1013, 1700
Juglandaceae
        *Carya alba (L.) Nutt. ex Ell.
        *Carya aquatica (Michx. f.) Nutt
        *Carya carolinae-septentrionalis (Ashe) Engl. & Graebn.
        *Carya cordiformis (Wangenh.) K. Koch

        Carya glabra (P. Mill) Sweet
        Carya laciniosa (Michx. f.) G. Don
        Carya ovata (Mill.) K. Koch
        Carya pallida (Ashe) Engl. & Graebn.
        Juglans nigra L.

Lamiaceae

§   *Lamium amplexicaule* L. 2056
        Lycopus virginicus L. 1895
        Monarda punctata L. 1304, 1345, 1468
        Physostegia virginiana ssp. *virginiana* (L.) Benth. 1805
        Prunella vulgaris L. 1179
        Pycnanthemum albescens Torr. & Grey 1874
        Salvia azurea Michx. ex Lam. 1549
        Salvia lyrata L. 986
        Scutellaria incana Biehler 1228
        Scutellaria integrifolia L. 1083
        Scutellaria ovata Hill 1150
        Teucrium canadense L. 1241
        Trichostema dichotomum L. 1416, 1494, 1566, 1668

Lauraceae

        Lindera benzoin (L.) Blume 1115, 1396
        Sassafras albidum (Nutt.) Nees 1875

Loganiaceae

        Gelsemium sempervirens 2046
        Mitreola petiolata (J.F. Gmel.) Torr. & Gray 1485
        Spigelia marilandica L. 1071, 1074

Lythraceae

        Ammannia coccinea Rottb. 1324, 1830
        Lythrum lineare L. 1832

Magnoliaceae

        Liriodendron tulipifera L.

Malvaceae

        Callirhoe triangulata (Leavenworth) Gray 1090
        Hibiscus laevis All. 1400
        Hibiscus moscheutos L. 1859
        Sida elliottii Torr. & Gray 1543, 1588A
        Sida spinosa L. 1504

Melastomataceae

        Rhexia virginica L. 1800

Meliaceae

§　　　* *Melia azedarach* L.

Menispermaceae

Calycocarpum lyonii (Pursh) Gray 1140, 1207, 1307

Cocculus carolinus (L.) DC. 1260, 1351

Menispermum canadense L. 1092

Molluginaceae

Mollugo verticillata L. 1306, 1536

Moraceae

§　　　* *Broussonetia papyrifera* (L.) L'Hér. ex. Vent.

* *Maclura pomifera* (Raf.) Schneid.

* *Morus rubra* L.

Nyctaginaceae

Mirabilis albida (Walt.) Heimerl 1146, 1250, 1352

Nyssaceae

* *Nyssa aquatica* L.

* *Nyssa biflora* Walt.

* *Nyssa sylvatica* Marsh.

Oleaceae

**Chionanthus virginicus* L.

Forestiera acuminata (Michx.) Poir. 1871

**Fraxinus americana* L.

**Fraxinus pennsylvanica* Marsh.

Onagraceae

Ludwigia alternifolia L. 1862, 1839

Ludwigia decurrens Walt. 1272A, 1281, 1534

Ludwigia glandulosa Walt. 1864

Ludwigia leptocarpa (Nutt.) Hara 1328

Ludwigia repens J.R. Forst. 1852

Oenothera biennis L. 1262, 1293

Oenothera fruticosa L. 1263

Oenothera laciniata Hill 947, 1037A

Oenothera rhombipetala Nutt. ex Torr. & Gray 1037, 1089

Oenothera speciosa Nutt. 1035

Oxalidaceae

Oxalis grandis Small 974

Oxalis stricta L. 951, 976, 1000, 1003, 1169, 1313, 1429, 1592

Oxalis violacea L. 952, 1683

Passifloraceae
        Passiflora incarnata L. 1252
        Passiflora lutea L. 1660, 1901
Phrymaceae
        Phryma leptostachya L. 1356
Phytolaccaceae
        Phytolacca americana L. 1075
Plantaginaceae
        Plantago aristata Michx. 1143, 1237
        Plantago rugelii Dcne.
        Plantago virginica L. 905, 946, 1041
Plantanaceae
        * *Platanus occidentalis* L.
Polemoniaceae
        Phlox divaricata L. 931
        Phlox pilosa L. 949, 951, 990, 1085
Polygalaceae
        Polygala grandiflora Walt. 1142, 1156, 1221
Polygonaceae
        Brunnichia ovata (Walt.) Shinners 1259, 1466
        Polygonum densiflorum Meisn. 1371
        Polygonum hydropiperoides Michx. 1208, 1372, 1439, 1644, 1677
        Polygonum lapathifolium L. 1171, 1444, 1600, 1823
        Polygonum pensylvanicum L. 1243, 1290, 1334, 1446
        Polygonum punctatum Ell. 1503, 1565
        Polygonum virginianum (L.) Raf. 1254
        Rumex hastatulus Baldw. 956, 1008
§   *Rumex crispus* L. 1015, 1054
Portulacaceae
        Claytonia virginica L. 909, 1682
§   *Portulaca oleracea* L. 1214, 1613
Primulaceae
        Lysimachia lanceolata Walt. 1317
Ranunculaceae
        Clematis crispa L.
        Clematis reticulata Walt. 1067, 1209, 1224, 1374
        Delphinium carolinianum Walt. 1195, 1704
        Ranunculus abortivus L. 1005, 1047

        Ranunculus hispidus var. *nitidus* (Chapman) T. Duncan 955,
           975
        Thalictrum thalictroides (L.) Eames & Boivin 2048

Rhamnaceae
        Berchemia scandens (Hill) K. Koch 1036
        Ceanothus americanus L. 1121
        Frangula caroliniana (Walt.) Gray

Rosaceae
        Agrimonia pubescens Wallr. 1266, 1508
        * *Amelanchier arborea* (Michx. f.) Fern
        Crataegus marshallii Egglest. 1686
        Crataegus monogyna Jacq. 1701
        Crataegus spathulata Michx. 1702
        Crataegus flava Ait. 1706
        Geum canadense Jacq. 1149, 1233
        Potentilla simplex Michx. 944, 984, 1135
        * *Prunus angustifolia* Marsh.
        * *Prunus caroliniana* (P. Mill.) Ait.
        Prunus mexicana S. Wats.
        * *Prunus serotina* Ehrh.
        * *Prunus umbellata* Ell.
        Rubus argutus Link 967, 994
        Rubus trivialis Michx. 908, 934

Rubiaceae
        Cephalanthus occidentalis L. 1350
        Diodia teres Walt. 1217, 1249, 1314, 1348, 1453
        Diodia virginiana L. 1170, 1246
        Galium aparine L. 1076
        Galium circaezans Michx. 1133, 1189
        Galium pilosum Ait. 1234, 1296
        Galium triflorum Michx. 954, 1123, 1225
        Galium uniflorum Michx 1187
        Houstonia longifolia Gaertn. 1050A
        Houstonia pusilla Schoepf 939, 971
        Mitchella repens L. 903, 1114
        Spermacoce glabra Michx. 1411, 1851

Rutaceae
        Ptelea trifoliata L. 1829
        * *Zanthoxylum clava-herculis* L.

Salicaceae
        *Populus deltoides Bartr. ex Marsh.
        *Salix nigra Marsh.
Santalaceae
        Comandra umbellata (L.) Nutt. 981
Sapindaceae
        Cardiospermum halicacabum L. 1460
Sapotaceae
        *Sideroxylon lycioides L.
Saururaceae
        Saururus cernuus L. 1112
Saxifragaceae
        Saxifraga virginiensis Michx. 919
Scrophulariaceae
        Agalinis purpurea (L.) Pennell 1472, 135A
        Aureolaria flava (L.) Farw. 1316
        Lindernia dubia (L.) Pennell 1028, 1274, 1282, 1325
        Lindernia dubia (Michx.) var. *anagallidea* Cooperrider 1017
        Leucospora multifida (Michx.) Nutt 1018, 1419
        Mecardonia acuminata (Walt.) Small 1577
        Mimulus alatus Ait. 1272, 1410
        Nuttallanthus canadensis (L.) D.A. Sutton 911, 930, 1144
        Pedicularis canadensis L. 989, 1053
        Penstemon digitalis Nutt. ex Sims 1065, 1141
        Scrophularia marilandica L. 1339
§   *Verbascum blattaria* L. 1801
§   *Verbascum thapsus* 1247
§   *Veronica arvensis* L. 970, 1688
        Veronica peregrina L. 922, 977
Solanaceae
        Physalis angulata L. 1442, 1604
        Physalis pubescens L. 1614
        Physalis virginiana P. Mill 1009, 1166, 1333
        Solanum americanum P. Mill 1257
        Solanum carolinense var. *carolinense* L. 1125, 1176
Symplocaceae
        *Symplocos tinctoria (L.) L'Hér.
Tiliaceae
        *Tilia sp.

Ulmaceae

　　　* *Celtis laevigata* Willd.

　　　* *Celtis tenuifolia* Nutt.

　　　* *Planera aquatica* J.F. Gmel

　　　* *Ulmus alata* Michx.

　　　* *Ulmus americana* L.

　　　* *Ulmus rubra* Muhl.

Urticaceae

　　　Boehmeria cylindrica (L.) Sw. 1248, 1311

　　　Laportea canadensis (L.) Weddell 1291, 1349

　　　Pilea pumila (L.) Gray 1461

Valerianaceae

　　　Valerianella radiata (L.) Dufr. 1038

Verbenaceae

　　　Callicarpa americana L. 1211

　　　Phyla lanceolata (Michx.) Greene 1177

　　　Stylodon carneus (Medik.) Moldenke 1068

§　　　*Verbena brasiliensis* Vell. 1391

　　　Verbena urticifolia L. 1340

Violaceae

　　　Viola affinis Le Conte 1001

　　　Viola bicolor Pursh 904, 972

　　　Viola walteri House 941, 983

Viscaceae

　　　Phoradendron leucarpum (Raf.) Reveal & M.C. Johnston 1684

Vitaceae

　　　Ampelopsis arborea (L.) Koehne 1242

　　　Ampelopsis cordata Michx. 1861

　　　Parthenocissus quinquefolia (L.) Planch. 1462

　　　Vitis rotundifolia Michx. 1091, 1402

　　　Vitis vulpina L. 1855

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FOSSOMBRONIA MARSHII (MARCHANTIOPHYTA), A NEW LIVERWORT SPECIES FROM ARKANSAS

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ABSTRACT

Field studies initiated by the third author in Arkansas, U.S.A. resulted in the discovery of an undescribed species of the liverwort genus *Fossombronia*. This new species is somewhat similar to *F. foveolata* Lindb., but is distinct in a suite of vegetative and reproductive characters. Among them is a much smaller plant size, an erect leaf stance, the capacity to produce apical tubers, dioecious sexuality, a different caulocalyx morphology, and fewer distal spore surface areolae. *Phytologia 92(2): 230-232 (August 2, 2010).*

KEY WORDS: Arkansas, *Fossombronia*, Marchantiophyta, new liverwort species.

During our field studies of the Fossombroniales Schljakov of the south-central United States, a new species of *Fossombronia* Raddi was discovered in the Western Gulf Coastal Plain of Arkansas. The following Latin diagnosis is given to make the name immediately available for use. A detailed treatment with illustrations of this taxon is in preparation.

FOSSOMBRONIA MARSHII J. R. Bray & Stotler, **sp. nov.**
Plantae dioicae, plantis masculinis parvioribus quam femineis, foliis erectis atque crispis, caulocalycibus stipitibus brevibus, apicibus

surculorum facientibus tubera; sporae areolatae, 3–5 areolis trans diametrum.

HOLOTYPE: U.S.A. ARKANSAS. Columbia County, Ebenezer Church Cemetery, 33° 35' N, 93° 17' W, on sandy soil in an open mowed grassy area, 10 March 1997, *Stotler & Crandall-Stotler 3940* (ABSH); PARATYPES: ARKANSAS. Columbia County, ca 1.8 mi W of Atlanta on Hwy 98, S side of Highway, 33° 12' N, 93° 05' W, on fine sandy loam along power line cut, 24 Nov. 1996, *Bray 297* (ABSH); 22 Nov. 1998, *Bray 327* (ABSH)

This species is named in honor of Daniel L. Marsh, Professor Emeritus of Biology, Henderson State University, Arkadelphia, AR whose field excursions in Arkansas throughout his teaching career were inspirational to his students.

Fossombronia marshii occurs on loose sandy to sandy loam soils that typically drain water fairly quickly and have little moisture holding capacity. While desiccation obviously could be a limiting factor of this habitat, apical tubers that can serve as perennnating structures are produced in this species. In contrast to *F. foveolata*, the plants are relatively small with an erect leaf stance and with leaves that are very rucked and crisped. The caulocalyx is short-stalked rather than long-stalked as in *F. foveolata* and the mouth is erect to incurved. The distal spore surface is areolate, with only 3 to 5 large areolae across the spore diameter, in contrast to the 5 to 7 areolae typically found in *F. foveolata*. *Fossombronia marshii* is the first dioecious species of this genus to be documented for North America. The plants are dimorphic, with male plants being much smaller than female plants. The antheridia are large, yellow to orange and crowded along the dorsal stem surface. Mature male plants are, therefore, strikingly conspicuous in the field, even without the aid of magnification. It was the observation of these very distinctive male plants that led to finding this species. Subsequent monitoring of the phenology of populations in several sites resulted in the discovery of female plants with mature sporophytes.

At present, *F. marshii* appears to be restricted to the Western Gulf Coastal Plains. Future field studies in Louisiana, Mississippi and

Texas and a review of herbarium specimens, particularly those labeled *F. foveolata*, likely will result in expanding the known distribution.

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CHROMOSOME COUNTS IN *BIDENS* SECTION, *GREENMANIA* (COREOPSIDEAE: ASTERACEAE)

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ABSTRACT

New chromosome counts are reported for *Bidens* section *Greenmania*. The majority of the counts are hexaploids and octaploids. *Phytologia 92(2): 233-240 (August 2, 2010)*.

KEY WORDS: *Bidens*, chromosome counts.

Chromosome numbers were made from mother cell squashes. Fresh mother cells were fixed in a modified Carnoy's solution: chloroform; absolute alcohol; glacial acetic acid (4:3:1 volume/volume). Young anthers were subsequently dissected and squashed in propio-carmine. Chromosome counts were independently confirmed by Dr. Thomas Melchert, Wayne Carlson, and/ or Tau San Chou (Botany Department, Univ. of Iowa, Ames, Iowa). Voucher specimens and drawings are deposited in the University of Texas Herbarium (TEX).

Mitotic counts of root tip material grown in the Univ. of Iowa greenhouse were made to supplement the meiotic data. Tips were pretreated in 8-hydroxy quinoline, fixed, washed and hydrolyzed in leuco-basic fuschin, cleared in acetic acid and squashed according to the methodology of Chen (1969).

Chromosome counts obtained in this study are listed in Table 1. The haploid number is listed in all cases. Counts obtained from root tips are marked with an asterisk (*).

At the start of this study, only two ploidy levels were known for *Bidens* sec. *Greenmania*: three diploid population (1. from Colima, Mexico (*B. reptans* var. *urbanii*, Keil & Stuessy, 1977), and (2. another

two diploid counts of *B. squarrosa* from Chiapas, Mexico (Solbrig, 1972). Three tetraploid populations of the latter had been found as well, one in Oaxaca, Mexico, and two from Guatemala (Robinson et al. 1981). Surprisingly, the great majority of populations examined were high polyploids: hexaploids or octaploids. Tetraploid populations were common in Guatemala, but not in Mexico. Only a small minority of the examined populations were diploid. The single dodecaploid (n = 72) population (of *B. boquetiensis*) was obtained in Panama. The cytological and other data (morphological and flavonoids) are discussed in more detail by Roseman (1986).

ACKNOWLEDGEMENTS

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Table 1. Chromosome counts of *Bidens* section *Greenmania*.

Country, State	Taxon Locality and collection number	n
	Bidens reptans	
JAMAICA:		
St. Thomas	Roseman 553, Union Hill, near Serge Island marble quarry.	12 12*
St. Catherine	Roseman 554, near Luidas Vale.	12 12*
	Bidens mexicana	
MEXICO:		
Guerrero	Roseman 515, 23 mi north of Acapulco, Hwy 95.	12
	Bidens antiguensis var. *antiguensis*	
GUATEMALA:		
Huehutetenango	Roseman 527, 5 mi northwest of Huehuetenango.	24
El Progresso	Roseman 600, 81 mi north of Santa Cruz Varapas on road to Coban.	24
Alta Verapas	Roseman 602, 20 mi west of Santa Cruz Varapas on Hwy 7W.	24
Quiche	Roseman 592, 4.8 mi north of Chichicastenango on road to Quiche.	24
	Roseman 529, 3.4 mi south of Chichicastenango.	24*
Solola	Roseman 591, 4.1 mi south of Panajachel.	24*
Sacatepequez	Roseman 585, 10.3 mi from Chimaltenango on road to Antigua.	24

Table 1. Chromosome counts of *Bidens* section *Greenmania* (cont'd).

Country, State	Taxon Locality and collection number	n
	Bidens antiguensis var. *antiguensis* (cont'd.)	
Escuintla	Roseman 571, 10 mi northwest of Esquintla on road to Guatemala City.	24
	B. antiguensis var. *procumbens*	
GUATEMALA:		
Zacapa	Roseman 594, 9 mi north of Zacapa-Isabal boundary.	24
	Roseman 597, near Juan de Paz, 3 mi from Zacapa-Izabal boundary.	24
	Bidens antiguensis var. *salvadorensi*	
GUATEMALA		
Quetzaltenango	Roseman 573, 12.3 mi southeast of Zunil, between Retalhuleu and Zunil.	24
	Bidens antiguensis x *B. squarrosa* (hybrid?)	
GUATEMALA:		
Retalhuleu	Roseman 572, 16.6 mi southeast of Zunil on road from Retalhuleu to Quetzaltenango.	60
	Bidens izabalensis	
GUATEMALA:		
Izabal	Roseman 598, 1 mi southwest of road to Mariscos from main highway to Puerto Barrios.	36
Zacapa	Roseman 595, near Juan de Paz, 4 mi from Izabal-Zacapa boundary.	36
	Bidens izabalensis x *B. squarrosa* (hybrid?)	
Alta Verapas	Roseman 601, just west of Santa Cruz Verapaz on road to San Cristobal	36 36*

Table 1. Chromosome counts of *Bidens* section *Greenmania* (cont'd).

Country, State	Taxon Locality and collection number	n
	Bidens squarrosa var. *squarrosa*	
COSTA RICA:		
Heredia	Roseman 566 north of Herredia on Hwy 9 near sawmill.	36
PANAMA:		
Cocle	Roseman 540, hills south of El Valle.	36
Veraguas	Roseman 537, above San Francisco, on road from Santiago to Santa Fe, about 12 mi south of Santa Fe.	36
	Bidens squarrosa var. *atrostriata*	
MEXICO:		
Oaxaca	Roseman 519, 46-47 mi south of Oaxaca just north of Totolapan.	36*
	Roseman 518, Monte Alban	36 36*
Michoacan	Roseman 514, 20 mi south of Arteaga.	36
	Bidens squarrosa var. *chiapensis*	
MEXICO:		
Chiapas	Roseman 525, 4 mi northwest of Comitan on Pan Americaan Hwy.	36
	Bidens squarrosa var. *tereticaulis*	
MEXICO:		
Veracruz	Roseman 613, 8-9 mi west of Minatitlan, Hwy. 180.	36
	Roseman 637, near Tampico, on highway from Temporal.	36

Table 1. Chromosome counts of *Bidens* section *Greenmania* (cont'd).

Country, State	Taxon Locality and collection number	n
	Bidens squarrosa var. *chapensis*	
St, Louis Potosi	Roseman 638, just south of Cuidad Valles on Hwy 85, near Rio Tempaon,	36
	Roseman 639 3-4 mi north of Tamazunchale	36*
Oaxaca	Roseman 616, 8 mi north of Chiltepec on Hwy 175 to Oaxaca.	36
	Roseman 621, 8 mi north of Valle, Nacional, Hwy 175.	36**
	Roseman 619, 7.8 mi north of Chiltepec on Hwy 175 to Oaxaca.	36
Hidalgo	Roseman 634, ca 12 mi south of Huehutla	36*
Yucatan	Roseman 609, 8-9 mi southwest of Izamal	36
	Roseman 611, Hwy 180 near turnoff to Chitzen-Itza.	36*
	Roseman 612, ruins at Uxmal	36 36*
	Bidens squarrosa var. *speciosa*	
GUATEMALA: Quetzalenango	Roseman 583, 1 mi from Zunil on road to Fuentes Georgina.	36
	Roseman 574, near Santa Maria de Jesus on road to San Lucas.	36
	Roseman 588, 8-9 mi from Panajachel on road to San Lucas.	36

Table 1. Chromosome counts of *Bidens* section *Greenmania* (cont'd).

Country, State	Taxon Locality and collection number	n
	Bidens squarrosa var. *speciosa*	
MEXICO: Vera Cruz	Roseman 639, 10 mi south of Huatusco, road to Fortin de las Flores.	36*
	Roseman 631, trail to upper falls, Cascades de Naolinca.	36*
	Bidens squarrosa var. *hondurensis*	
COSTA RICA: Puntarenas	Roseman 570, 6 mi south of Santa Elena, road from Monteverde.	36
	Roseman 569, 5 mi south of Santa Elena road from Monteverde.	36
	B. squarrosa var *hondurensis* x *B. boquetiensis* (hybrid?)	
COSTA RICA: Punarenas	Roseman 568, 3 mi south of Santa Elena road from Monteverde.	36**
	Bidens boquentiensis	
PANAMA: Chiriqui	Roseman 556, 23 mi north of church at Boquet, road to Altos de Boquete.	48*
	Roseman 555, ca 3 mi north of Boquete, near bridge.	72 72*
COSTA RICA: San Jose	Roseman 532, Cerro de la Muerte, ca 65 mi north of San Isidro.	48

Table 1. Chromosome counts of *Bidens* section *Greenmania* (cont'd).

Country, State	Taxon Locality and collection number	n
	Bidens boquentiensis (cont'd)	
	Roseman 536, Panamerican Hwy, ca 6 mi north of San Isidro.	48
	Roseman 561, Cerro de la Muerte, ca 60 mi north of San Isidro.	48*
Cartago	Durkee 573-22, Tapanti	48*
Puntarenas	Roseman 567, 1.5 mi of cheese factory, Monteverge.	48*
Heredia	Roseman 563, Vara Blanca	36
	Bidens holwayi	
GUATEMALA:		
Quetzaltenango	Roseman 576, western slopes of Volcan Zunil, on road from Zunil to Fuentes Georgina.	48
Quiche	Roseman 605, 5 mi south of Nebaj on road to Sacapulas.	48
	Roseman 606, 7 mi south of Nebaj on road to Sacapulas.	48
	Roseman 608, between km 10 and km 11, south of Nabaj on road to Sacapulas.	48

*   Haploid chromosome numbers are listed. Numbers with an asterisk are one-half of the mitotic chromosome number obtained from root tips; all other numbers are meiotic counts.

**   Abnormal meiosis, circle of chain of four chromosomes.

KEYS TO THE FLORA OF FLORIDA -- 25, LEMNACEAE

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ABSTRACT

The Lemnaceae in Florida are represented by 4 genera: *Lemna*, with 5 species; *Spirodela*, with 2 species; *Wolffiella*, with 2 species; and *Wolffia*, with 3 species. Of these, 6 are non-native, and one is ranked as invasive. None is endemic. Three species of *Lemna* and one of *Wolffiella* are excluded. The use of *Spirodela oligorrhiza* is briefly justified. The segregate genus *Landoltia* is seen as unneeded. An amplified key is given to the Florida taxa. *Phytologia 92(2): 241-248 (August 2, 2010).*

Species of the Lemnaceae, the duckweeds, are omnipresent members of the Florida wetlands flora. They float on or just below the surface of quiet waters, frequently as scattered plants, at times as exceedingly abundant masses. Often two or more species are found intermixed. And often -- perhaps because of their individually insignificant size, perhaps because of their reputed resistance to ready identification -- they are little noted and too little collected.

Early systematic knowledge of the Lemnaceae was brought together by Friedrich Hegelmaier of Germany, first by studies of the species of Brazil (1868), later by a world monograph of the family (1878, 1895). A more narrowly focused view of the American duckweeds was produced by Charles H. Thompson with his revision of the Lemnaceae of North American north of Mexico (Ann. Rep. Missouri Bot. Gard. 9: 1-21. 1898). Thompson recognized 7 species of *Lemna* (one consisting of 2 varieties), 2 species of *Spirodela* (one only as a footnote), 3 species of *Wolffiella*, and 3 species of *Wolffia*. With the exception of one species recognized later (*L. obscura*, raised from

varietal rank under *L. minor*), Thompson's study is quite similar to modern understanding of the genus. His detailed descriptions and his approximate ranges, as documented by citation of the many specimens examined, underlay all more recent work. Only a few changes in nomenclature, necessitated by examination of types then unavailable, has consequentially disturbed his presentation.

In the 1960s interest in the Lemnaceae was stimulated by Edwin H. Daubs (Ill. Biol Monogr. 34: 1-118. 1965) who attempted a revision of Hegelmaier's world monograph. [With uneven success; note a critical review: H. L. Clark & J. W. Thieret (Sida 2: 437-438. 1966).] For purposes of the present study, Daubs' strength lay in his personal knowledge of plants in the field, including Florida.

The 1960s also marked the rise in Europe of two centers of study of the Lemnaceae. In the Netherlands, C. den Hartog & F. van der Plas undertook a careful documentation of the classification of the family, with inclusion of new species and newly available flowering and fruiting structures, summarized by their comprehensive synopsis (Blumea 18: 355-368. 1970). And in Switzerland, Elias Landolt began his own exhaustive studies, including morphology, classification, distribution, even nutritional requirements, with a series of papers that perhaps equal if not surpass the level of understanding elsewhere attained of any plant family, culminating in his masterful two-volume "The Family of Lemnaceae" (1986).

Lack of early collection records for most Florida Lemnaceae gives uncertainty to the judgment of which species are native and which are introduced. Those considered here to be introduced are *Lemna minuta*, *Lemna minor*, *Spirodela oligorrhiza*, *Wolffiella oblonga*, *Wolffia brasiliensis*, and *Wolffia globosa*. Each has a limited Florida distribution or is disjunct from its range elsewhere. Of these, only *S. oligorrhiza* carries documentation as to its approximate date and possible mode of arrival. This species was first identified in the United States in Missouri where it was suspected to have been imported with goldfish (A. Saeger, Bull. Torrey Bot. Club 61: 233-236. 1934). The earliest Florida collection seen was in 1955. By 1961 it was found well established in 6 locations in peninsular Florida (E. H. Daubs, Rhodora 64: 83-85. 1962). Florida has an active tropical fish industry with

frequent imports, which may be the pathway by which these species have reached the state.

Even though previous studies of the Lemnaceae seem wholly comprehensive, a matter of nomenclature and one of classification requires attention. Landolt (1980, 1986, 2000) and others have treated *Spirodela oligorrhiza* under the name *S. punctata* (Meyer) Thompson. The basionym, *Lemna punctata* G. F. W. Meyer (1818), is of a spm. (GOET, now lost) from the Essequibo River, Guiana. Thompson (1898) equated (but did not neotype) Meyer's description of *L. punctata* with a collection labeled as from Tierra del Fuego (where no Lemnaceae are known to modern workers). This plant, now widely introduced in the American Southeast, is of Asian or Australian origin (Landolt, 1986), not South American. The possibility is remote that Meyer's plant from tropical Guiana could have been the same as the one (as Thompson believed) from frigid southern Patagonia. The Meyer specimen must have been of *S. intermedia* W. Koch (1932), the only one of these two species that is native and widespread in South America (incl. Guiana). With Thompson's error corrected, and with Meyer's *L. punctata* seen as conspecific with *S. intermedia*, the earliest name for the Asian introduction to southeastern North America is *Spirodela oligorrhiza* (Kurz) Hegelm. (basionym: *Lemna oligorrhiza* Kurz, 1867). This name was used by Daubs (Rhodora 64: 83-85. 1962) in the first report of its presence in Florida. This argument in greater detail, including apparent origin of the mislabeled Tierra del Fuego spm., is given elsewhere (Ward, in press). [As a corollary, the common South American plant must take on the name *S. punctata* (Meyer) Thompson, and *S. intermedia* W. Koch is reduced to synonymy.]

Spirodela oligorrhiza (or *S. punctata*, sensu Thompson) has recently been distinguished at generic level from other *Spirodela*, as *Landoltia punctata* (Meyer) Les & Crawford (Novon 9: 530-533. 1999). DNA data indeed appear to place *S. oligorrhiza* (with its several roots per plant) somewhat closer to *Lemna* (with its single root) than to *S. polyrrhiza* (also with several roots). Yet no harm will result if the morphologically similar (if possibly cladistically questionable) several-rooted species are kept together. If this species were confidently seen as closer to *Lemna* than to *Spirodela*, the proper action would have been to use the pre-existing name, *Lemna punctata* Meyer (1818),

rather than form a new single-species genus. It is noted that Elias Landolt, the dean of modern students of the Lemnaceae, never (in a paper wholly of his own writing) divided *Spirodela* (cf. Flora N. Amer. 22: 145. 2000).

The present study, limited to Florida plants, attempts to bring into sharper focus the 12 species of Lemnaceae found within the state. Most encouraging for the field botanist or environmentalist who has been tempted to pass over the duckweeds with a simple "*Lemna sp.*" or an inaccurate "*Lemna minor*," their identification can be relatively straightforward. First, disregard those names whose presence in the state are reported in error. Second, dismiss as improbable the two species of *Lemna* and the one of *Wolffia* which, though reliably reported, are of great rarity. Third, recognize that the species of *Spirodela* and *Wolffiella*, two each, and the two remaining species of *Wolffia* are appreciably different from one another in gross form and can be readily distinguished. Thus remaining are 3 species of *Lemna* that themselves can be separated by careful inspection. Any red-purple coloration indicates *L. obscura*. One vein in frond suggests *L. valdiviana*. And 3 veins points to *L. aequinoctialis*.

LEMNACEAE Dumort.   Duckweeds [1]

1. Plants with roots; with two lateral reproductive pouches.

  2. Roots one per plant.

    3. Upper surface of fronds flat, without papillae; fronds narrowly elliptic to ovate, very small (often <1.0 mm. long); veins one (or none apparent).

      4. Fronds narrowly elliptic, somewhat asymmetric; plants usually in attached groups of 3-7. Floating or submersed aquatic herb. Quiet ponds, ditches. Throughout; frequent. Spring-summer. [*Lemna cyclostasa* (Ell.) Thompson]

                              Lemna valdiviana Phil.

4. Fronds obovate, symmetric; plants usually separate or attached in pairs. Floating aquatic herb. Cool swamps. Central panhandle (Jackson, Leon counties); rare. Spring-summer. [*Lemna minima*, misapplied; *Lemna minuscula* Herter]
                                        * **Lemna minuta** HBK.

3. Upper surface of fronds flat or convex, with papillae; fronds elliptic to obovate or orbicular, small (usually >1.5 mm. long); veins 3 (in some indistinct).

   5. Sheath winged at base of root; fronds thin, translucent, with 3 distinct veins. Floating aquatic herb. Canals, ditches, quiet ponds. Throughout; common, at times locally profuse. Spring-summer. [*Lemna trinervis* (Austin) Small]
                              Lemna aequinoctialis Welw.

   5. Sheath cylindrical at base of root, without wings; fronds opaque, with 3 sometimes indistinct veins, shiny on upper surface.

      6. Lower surface of fronds dark red-purple, convex; upper surface with a single evident papilla near apex. Floating aquatic herb. Warm water of ditches, shallow ponds. Throughout; common, often exceedingly abundant in nutrient-rich cattle ponds. Summer-fall. [*Lemna gibba*, misapplied; *Lemna minor* var. *obscura* Austin]
         SOUTHERN DUCKWEED   **Lemna obscura** (Austin) Daubs

      6. Lower surface of fronds green to faintly red-purple, flat to convex; upper surface with several indistinct papillae in line near apex. Floating aquatic herb. Ditches. North Florida (Columbia Co.); rare. Summer. [*Lemna minima* Thuill. ex Beauv.]
                                        * **Lemna minor** L.

2. Roots two or more per plant.

   8. Plants with 5-10 roots; fronds 2.5-8.0 mm. wide, with 5-15 prominent veins. Floating aquatic herb. Cypress swamps, shaded ponds, spring runs. Throughout; common. Spring-summer.
GREATER DUCKWEED   **Spirodela polyrrhiza** (L.) Schleid.

   8. Plants with 2-5 roots; fronds 1.5-3.0 mm. wide, with 3-5 obscure veins. Floating aquatic herb. Ditches, stream margins, swamps. Throughout; very common, often profusely covering water surface. Spring-summer. Invasive (first record: Dunedin, Pinellas Co., 1955). [*Spirodela punctata*, misapplied; *Landoltia punctata*, misapplied].
LESSER GREATER DUCKWEED.
                          * **Spirodela oligorrhiza** (Kurz) Hegelm.

1. Plants without roots; with one basal reproductive pouch.

   9. Fronds flat, thin, appearing two-dimensional, >4 times longer than wide.

     10. Fronds 0.4-0.7 mm. wide, >9 times as long as wide, scimitar-shaped. Submersed aquatic herb. Floating just below surface of quiet waters. Throughout; frequent. Spring-summer-fall. [*Wolffiella floridana* (J. D. Sm.) Thompson]
                           Wolffiella gladiata (Hegelm.) Hegelm.

     10. Fronds 0.5-1.5 mm. wide, <8 times as long as wide; tapered-oblong. Floating or submersed aquatic herb. Swamps, canals. Peninsula (Collier to Alachua counties), scattered to central panhandle (Wakulla Co.); infrequent. Spring-summer-fall.
                         * **Wolffiella oblonga** (Phil.) Hegelm.

   9. Fronds ellipsoidal, strongly biconvex, three-dimensional, <2 times as long as wide.

11. Frond with sharply defined upper and lower surfaces, the upper supporting a blunt conical mound arising from flat surface, the lower strongly convex (plants thus ± boat-shaped); brown pigment cells apparent within tissue. Minute floating aquatic herb. Ditches, ponds. Across panhandle (Escambia, Jackson, Leon counties), north Florida; infrequent. Spring-summer. Fronds appear to have cuticularized upper surface, thus float dry. [*Bruneria punctata* (Griseb.) Nieuwl.; *Wolffia papulifera* Thompson; *Wolffia punctata* Griseb.]

* **Wolffia brasiliensis** Wedd.

11. Frond with confluent upper and lower surfaces, hemispherical (plants prolate-spheroidial or football-shaped); dark pigment cells absent.

12. Fronds subspheroidal, 1.0-1.3 times as long as broad, >0.4 mm. wide. Minute floating or submersed aquatic herb. Ponds, ditches, spring-fed streams. Throughout; frequent (infreq. in south peninsula). Spring-summer. Fronds not cuticularized, thus float below meniscus. [*Bruneria columbiana* (Karst.) Nieuwl.]
WATERMEAL   **Wolffia columbiana** Karst.

12. Fronds ellipsoidal, 1.3-2.0 times as long as broad, <0.5 mm. wide. Minute floating aquatic herb. Ditches. West-central peninsula (Pinellas Co.); rare. Summer.

* **Wolffia globosa** (Roxb.) Hartog & Plas

Excluded names:

Lemna gibba L.
Reported for southeast U.S. (H. L. Clark, in Godfrey & Wooten, 1979), but restricted to areas westward (Landolt, Flora N. Amer. 22:147. 2000). Many plants so identified are of *L. obscura*.

Lemna perpusilla Torr.
Northern. Reported for Florida (Small, 1933; Godfrey & Wooten, 1979), apparently by confusion with *L. aequinoctialis*.

Lemna trisulca L.   Star Duckweed
Northern. Reported for Florida (Daubs 1962, 1965). Not known south of Virginia (Landolt, 2000).

Wolffiella lingulata (Hegelm.) Hegelm.
Reported as "rare, Glades Co." (Landolt, 1980). Not found again. Best seen as an ephemeral introduction.

NEW AND NOTEWORTHY PLANTS OF TEXAS

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ABSTRACT

Rhexia alifanus (Melastomataceae) and *Rhynchospora inundatum* (Cyperaceae), both previously reported in the state, are documented as part of the flora of the state. *Ratibida pinnata* (Asteraceae), *Tradescantia fluminensis* (Commelinaceae) and *Saccharum ravennae* (Poaceae) are reported as new to Texas. *Phytologia 92(2): 249-255 (August 2, 2010).*

KEY WORDS: Texas, *Rhexia*, Melastomataceae, *Rhynchospora*, Cyperaceae, *Ratibida*, Asteraceae, *Tradescantia*, Commelinaceae, *Saccharum*, Poaceae.

The following two species, both previously mentioned as occurring in Texas, are documented as part of the flora of the state. These are followed by the report of three species new to the state.
Rhexia alifanus Walter (Melastomataceae)
Specimen cited: **U.S.A.** **TEXAS. Newton Co.:** Hancock Management Land, 2.1 miles E of junction of Hwy 363 and Big Cow Creek on Hwy 363, then 0.2 mile south; flatwoods pond with *Rhynchospora perplexa, R. filifolia, R. elliottii, R. gracilenta, R.*

cephalantha, Eleocharis equisetoides, Dichanthelium scabriusculum, Eriocaulon decangulare, Xyris latifolia, and *Rhexia lutea,* 23 July 2008, *J. Singhurst 16123* (BAYLU).

      Rhexia alifanus is characterized by glabrous stems, curved anthers 5–11 mm long, and seeds 1.2–1.4 mm long. The species is distributed from North Carolina, south to Florida, then west to Louisiana and Texas. The history of the citation of *R. alifanus* in Texas lacks verification (a voucher specimen). It was first mentioned as occurring in the state by Kral and Bostick (1969). However, the species was not included in the *Manual of the Vascular Plants of Texas* by Correll and Johnston (1970), possibly due to the short period of time between the two publications. In 1972, Correll and Correll included the species in *Aquatic and Wetland Plants of Southwestern United States,* stating "in s.e. Tex. (Hardin Co.)" while Brown (1972) also mentioned the occurrence of the species in Texas. The species was included in the second printing of Correll and Johnston (1979) with the comment "recently reported from Hardin Co., s.e. Tex." Since then, the species has been included as part of the state's flora and is listed in Hatch et al. (1990), Johnston (1990), Jones et al. (1997), Turner et al. (2003), and USDA, NRCS (2010).

      Substantial effort has failed to locate either a voucher specimen or a reference citing a specimen from the state. Under such a circumstance, it appears proper to cite the above specimen to document the occurrence of the species within the state. The species is considered to be native to Texas.

Rhynchospora inundata (W. Oakes) Fern. (Cyperaceae)
Specimens cited: U.S.A.   Texas.   Newton Co.: E of Sandhill Cemetery, deep peat pond with floating mats, 24 Sep 2009, *J. Singhurst 18003* (BAYLU); **Robertson Co.:** Love Ranch bog, 15 miles east of New Baden, mud and swampy soil, frequent, 2 Jul 1952, *S. K. Menon 325* (TAES); **Washington Co.**: Roadside ditch on Hwy 290, 4.2 mi. SE of county line; sandy soil, 29 Jun 1982, *James Kessler 6197* (TAES).

      The initial mention of *Rhynschospora inundata* in Texas is in Jones' et al. (1997) checklist of vascular plants of the state. No indication of importance (i.e., was the first mention of the species in the

state) nor was a listing of representative specimens presented. This report was used by USDA, NRCS (2010) as the basis for including Texas as part of the distribution of the species. The distribution of the species presented in Kral (2002) does not include Texas and Turner et al. (2003) do not include the species in their atlas of the vascular flora of the state. The specimens cited above thus serve as documentation of the species in Texas.

The species is distributed in the lower Atlantic and Gulf coastal plains from Massachusetts and Rhode Island to Florida and west to eastern Texas. It is considered to be native to the state. This emergent species occurs in savannah ponds and pools. Within Texas, the species is known from widely separated locations. The Robertson Co. location is approximately 280 km west of the Newton Co. location, while the Washington Co. location is 240 km west of the Newton Co. record. The Robertson Co. and Washington Co. locations are about 90 km distant. Kral (2002) includes Louisiana within the distribution of *R. inundata*, but neither Thomas and Allen (1993) nor USDA, NRCS (2010) indicate the presence of the species in that state.

Label data from Texas specimens suggest that the low representation of the species in Texas herbaria may be related to misdetermination as *R. corniculata*, (Lamarck) A. Gray, a similar and much more abundant cespitose species lacking slender scaly rhizomes characteristic of *R. inundata*. Additional information is in Kral (2002).

Ratibida pinnata (Ventenat) Barnhart (Asteraceae)
Specimen Cited: U.S.A. Texas. Bowie Co.: Godley Prairie, 1.8 mi. S of FM 1840 and Hwy 98, just S of Godley Community, alfisol prairie with mima mounds, 27 Jun 2009, *J. Singhurst 17500* (BAYLU).

Ratibida is a genus of seven species, four of which occur in the United States and Canada (Urbatsch and Cox, 2006). Until now, three species have been recorded in Texas. These are *R. columnifera* (Nuttall) Wooton and Standley, widely distributed in the eastern two-thirds if the state, *R. peduncularis* (T. & G.) Barnhard of the south Texas plains and the Gulf prairies and marshes, and *R. tagetes* (E. James) Barnhard of the western third of Texas. *Ratibida pinnata*, here reported as new to Texas, is widely distributed in eastern North

America from west and south of the Appalachian Mountains to the eastern portions of the tall grass prairies of the Great Plains (Urbatsch and Cox 2006). The species is reported in Choctaw and Mc Curtain counties, Oklahoma, which border Texas, and Hempstead and Clark counties, in southwest Arkansas (USDA, NRCS 2010). The Texas location cited is about four miles southwest of New Boston, Bowie Co., Texas. A key to the species of *Ratibida* and an illustration of *R. pinnata* are in Urbatsch and Cox (2006). The species is considered to be native to the state.

Tradescantia fluminensis Vellozo (Commelinaceae).
Specimens cited: U.S.A. Texas. Travis Co.: Austin, jct. of Koenig Ln. and Waller Creek, 19 Mar 2009, J. & A. *Singhurst 17671* (BAYLU); Austin, jct. 34th Street and Shoal Creek, 25 Jun 2009, *J. Singhurst 17515* (BAYLU); **Victoria Co.:** Victoria, 12 May 1968, *Tim Edwards s.n.* (BAYLU).

        Tradescantia fluminensis is native to South America, where it is distributed from Brazil to Argentina. The species is naturalized in coastal California and the Gulf Coastal plain of Alabama, Florida, and Louisiana (Faden 2000). The species has recently been reported in Georgia by Carter et al. (2009). Reports of the species by Small (1933) in North Carolina and Georgia are unconfirmed (Faden 2000). USDA, NRCS (2010) mentions that the species occurs in Kentucky. In Texas, the species occurs in shaded areas, springs, streams, and seeps in limestone, often in mats.

Saccharum ravennae L. (Poaceae)
Specimen cited: U.S.A. Texas. Hemphill Co.: Gene Howe Wildlife Management Area, north side of the Canadian River, 15 Oct 2009, *J. Singhurst* 17613 (BAYLU). **Wheeler Co.:** 2.6 mi. N of Shamrock (jct. of Interstate Hwy 40 and U.S. Hwy 83), north side of North Fork Red River, west side of Hwy 83, 15 Oct 2009, *J. Singhurst 17670* (BAYLU).

        Saccharum ravennae (formerly *Erianthus ravennae* Beauv.) is a native of southern Europe and western Asia (Webster 2003). Bailey (1949) mentions that the species is utilized as an ornamental, especially were thermal regimes are too severe for pampas grass (*Cortaderia*

selloana (Schult & Schult. f.) Asch. & Graebn.). The species is first mentioned as occurring in the United States in Hitchcock (1935, 1950), but is unnumbered and does not appear in the key. In that work, such plants were not considered as permanent constituents of the flora and appear not to be established. However, it is mentioned in both editions that the species is "Established along irrigation ditches near Phoenix, Arizona." The species was reported as adventive in Arizona by Kearney and Peebles (1960). The oldest herbarium specimen located was from Fresno, California, collected 4 August 1918 by *P.B.Kennedy s.n.* (CDA 206). It is not known if this was a naturally occurring or cultivated plant. Webster (2003) mentions that the species occasionally escapes from cultivation and may persist. Presently, *S. ravennae* has been recorded in a discontinuous distribution as far east as Florida, north to Maine and Michigan and west to California. The occurrence of the species in Texas should be monitored to determine if it will become a permanent part of the state's flora and possibly a serious invasive pest.

ACKNOWLEDEMENTS

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THE LEAF ESSENTIAL OIL OF *JUNIPERUS ZANONII* AND COMPARISIONS WITH THE OILS OF *J. JALISCANA, J. MONTICOLA* AND *J. SALTILLENSIS*

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ABSTRACT

The first report on the composition of the leaf essential oil of *Juniperus zanonii* is presented and the oil compared to the oils of closely related species: *J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis and J. saltillensis*. The leaf oil of *J. zanonii* is dominated by sabinene (30.9%), terpinen-4-ol (13.5%) and manoyl oxide (11.7%) with moderate amounts of γ-terpinene (5.6%), citronellal (4.3%), and abietatriene (3.4%). *Juniperus saltillensis* oil is dominated by camphor (44.5%) and α-pinene (12.1%) with moderate amounts of sabinene (7.7%), limonene (2.6%) and camphene hydrate (2.2%). The oils of the monticolan junipers (*J. m.* f. *monticola, J. m* f. *compacta, J. m.* f. *orizabensis*) are dominated by bornyl acetate (34.8, 44.2, 10.7%) and contain three compounds not found in other taxa in this study: δ-2-carene, linalyl acetate and methyl citronellate. However, these three forms have a surprising amount of variation among them. The oil of *J. jaliscana* is dominated by α-pinene (49.5%), limonene (15.1%) and β-phellandrene (10.0%). *Phytologia 92(2): 256-265 (August 2, 2010)*.

KEY WORDS: *Juniperus zanonii, J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis, J. saltillensis*, leaf essential oil composition, Cupressaceae, terpenes.

Recently, Adams et al. (2010) recognized the shrubby subalpine juniper from Cerro Potosi and adjacent peaks as a new species, *Juniperus zanonii* R. P. Adams, distinct from the *J. monticola* Mart. f. *compacta* Mart. from the trans-volcanic region of central Mexico. This was based in part on analyses of nrDNA and trnC-trnD sequences (Adams 2008) of the serrate leaf margined junipers of the western hemisphere that indicated the closest relative to the *J. zanonii* was *J. saltillensis* M. T. Hall (Fig. 1), not *J. monticola* Mart. In fact, *J. monticola* appears in a clade with *J. jaliscana* Mart. (Fig. 1).
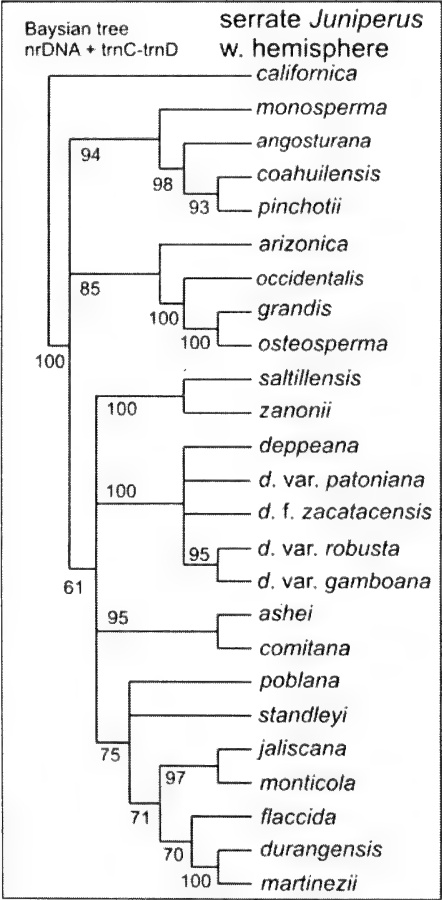

Figure 1. Bayesian tree for the serrate-leave *Juniperus* of the western hemisphere (modified from Adams, 2008) based on nrDNA + trnC-trnD. *J. zanonii* is in a well supported clade with *J. saltillensis*, whereas *J. monticola* (f. *monticola*) from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*.

A comparison of *J. zanonii* with *J. jaliscana, J. monticola f. monticola, J. monticola f. compacta and J. saltillensis* using 27 SNPs from nrDNA and petN-psbM (Adams et al., 2010) revealed (Fig. 2) that *J. zanonii* is not closely related to *J. monticola* f. *compacta* (from Pico Ixtaccihuatl) and that plants of *J. m.* f. *monticola* and f. *compacta* could not be resolved (Fig. 2) using these sequences.
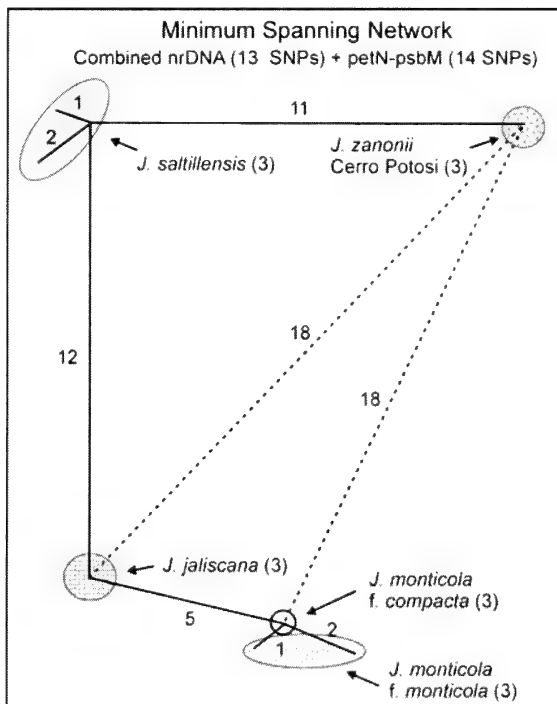

Figure 2. Minimum spanning network based on 27 SNPs from nrDNA and petN-psbM. Adapted from Adams et al. (2010).

The compositions of the leaf essential oils of the related taxa have been reported: *J. jaliscana* (Adams, Zanoni and Hogge, 1985); *J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis* (Adams et al. 1980a) and *J. saltillensis* (Adams et al. 1980b). A summary of the compositions of the leaf essential oils of the serrate junipers of the western hemisphere has been presented by Adams (2000). It is worth

noting that many unknown compounds reported in these early papers are now indentified in this updated examination of the aforementioned oils.

The purpose of the present study is to present the first analysis of the leaf essential oil of *J. zanonii* and compare its composition with that of closely related species: *J. jaliscana, J. monticola* f. *monticola, J. m.* f. *compacta, J. m.* f. *orizabensis and J. saltillensis*.

MATERIALS AND METHODS

Specimens collected: *J. jaliscana, Adams 6846-6848*, 12/12/1991, 940 m, 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. monticola* f. *compacta*: *T. A. Zanoni 2601-2618,* Pico Ixtaccihuatl, Mexico; *J. monticola* f. *monticola, Adams 6874-6878*, 12/20/1991, 2750 m, El Chico National Park, Hidalgo, Mexico; *J. monticola* f. *orizabensis*: *T. A. Zanoni 2627-2636*, Pico de Orizaba, Vera Cruz, Mexico; *J. saltillensis, Adams 6886-6890*, 12/21/1991, 2090 m, on Mex. 60, 14 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. zanonii, Adams 6898-6902*, 12/21/1991, 3490 m, Cerro Potosi, Nuevo Leon, Mexico. Voucher specimens are deposited at BAYLU.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The compositions of the leaf oils are shown in Table 1. The leaf oil of *J. zanonii* is dominated by sabinene (30.9%), terpinen-4-ol (13.5%) and manoyl oxide (11.7%) with moderate amounts of γ-terpinene (5.6%), citronellal (4.3%), and abietatriene (3.4%).

Juniperus saltillensis leaf essential oil is dominated by camphor (44.5%) and α-pinene (12.1%) with moderate amounts of sabinene (7.7%), limonene (2.6%) and camphene hydrate (2.2%).

The oil of *J. jaliscana* is dominated (Table 1) by α-pinene (49.5%), limonene (15.1%) and β-phellandrene (10.0%) with moderate amounts of myrcene (4.5%) and β-pinene (2.3%).

The oils of the monticolan junipers (*J. m.* f. *monticola, J. m* f. *compacta, J. m.* f. *orizabensis*) are dominated by bornyl acetate (34.8, 44.2, 10.7%) and contain three compounds not found in other taxa in this study: δ-2-carene, linalyl acetate and methyl citronellate. The oil of *J. m. f. monticola* also contains moderate amounts of α-pinene (15.1%), limonene (6.7%), β-phellandrene (6.8%) and elemol (5.4%), whereas f. *compacta* contains moderate amounts of sabinene (17.8%), β-phellandrene (6.3%), terpinen-4-ol (9.8%), elemol (5.7%) and manoyl oxide (6.3%) and f. *orizabensis* has α-pinene (6.5%), limonene (7.9%), β-phellandrene (7.8%) and camphor (4.4%). Despite the seemingly small amount of variation seen in the DNA sequences for nrDNA and petN-psbM (Fig. 2), these three forms have a surprising amount of variation in their leaf essential ols.

It is interesting that the oil of *J. m.* f. *compacta* shares some similarity to *J. zanonii*. Note the similar amounts of sabinene, terpinen-4-ol, manoyl oxide and abietatriene (Table 1). However, *J. m* f. *compacta* and *J. zanonii* differ considerably in the concentrations of borneol, bornyl acetate, β-phellandrene, citronellal and many trace components (Table 1).

ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf essential oils of *J. jaliscana* (Jal), *J. monticola* f. *monticola* (MM), *J. m.* f. *compacta* (MC), *J. m.* f. *orizabensis* (MO), *J. saltillensis* (Salt) and *J. zanonii* (Zan). Compounds in bold appear to separate the taxa. t = trace, < 0.1%, RI = retention index on DB-5. Unidentified compounds have the four strongest ions listed.

RI	Component	Salt	Zan	MC	MO	MM	Jal
846	(E)-2-hexenal	-	-	-	0.3	-	0.1
846	(E)-3-hexenol	-	-	-	-	-	0.6
849	ethyl isovalerate	-	0.2	-	-	0.1	-
921	tricyclene	0.4	-	0.2	0.9	1.0	0.1
924	α-thujene	0.3	1.8	0.4	-	-	t
932	**α-pinene**	**12.1**	**3.0**	**4.5**	**6.5**	**15.1**	**49.5**
945	α-fenchene	-	-	-	-	-	t
946	camphene	0.8	t	0.3	1.8	1.4	0.5
961	verbenene	1.8	0.2	t	-	1.8	0.1
969	**sabinene**	**7.7**	**30.9**	**17.8**	**t**	**t**	**0.1**
974	β-pinene	1.3	0.6	0.5	0.2	0.9	2.3
988	myrcene	1.9	1.6	1.5	2.4	3.0	4.5
1001	**δ-2-carene**	-	-	**0.7**	**2.3**	**0.1**	-
1002	α-phellandrene	t	0.3	t	0.4	0.1	t
1008	δ-3-carene	0.4	-	0.2	0.2	t	t
1014	α-terpinene	0.5	0.3	1.2	0.2	0.1	t
1020	p-cymene	0.1	0.2	0.8	0.7	0.2	0.1
1024	**limonene**	**2.6**	**1.0**	-	**7.9**	**6.7**	**15.1**
1025	**β-phellandrene**	**1.3**	**1.1**	**6.3**	**7.8**	**6.8**	**10.0**
1026	1,8-cineole	0.1	-	-	-	-	-
1044	(E)-β-ocimene	0.1	-	-	0.1	0.2	0.2
1054	**γ-terpinene**	**0.8**	**5.6**	**2.4**	**0.4**	**0.6**	**0.4**
1063	unknown, 88,101,43,158	0.2	-	-	0.7	1.0	-
1065	**cis-sabinene hydrate**	**0.3**	**0.9**	**1.0**	-	**t**	-
1067	cis-linalool oxide (furanoid)	-	-	0.1	t	-	-
1086	terpinolene	0.7	1.7	-	0.7	0.8	1.1
1092	C10-OH, 96,109,137,152	2.8	-	-	-	0.6	-
1095	linalool	0.6			1.4	1.1	0.2
1098	trans-sabinene hydrate	-	1.0	1.3	-	-	-
1100	n-nonanal	-	0.1	-	t	-	t
1102	isopentyl-isovalerate	-	0.1	0.1	-	-	-
1112	3-methyl-3-buten-methyl-butanoate	-	0.2	0.2	-	0.2	t
1114	endo-fenchol	-	-	-	-	-	t
1118	**cis-p-menth-2-en-1-ol**	**0.2**	**0.8**	**0.8**	-	**0.1**	**t**

RI	Component	Salt	Zan	MC	MO	MM	Jal
1122	α-campholenal	-	-	-	t	-	0.1
1135	trans-pinocarveol	-	-	-	-	-	0.1
1136	trans-p-menth-2-en-1-ol	-	0.5	0.5	0.9	0.1	-
1141	**camphor**	**44.5**	**0.1**	**1.6**	**4.4**	**1.0**	**0.1**
1144	neo-isopulegol	-	0.3	-	-	0.4	-
1145	camphene hydrate	2.3	t	0.2	1.4	1.0	0.1
1148	**citronellal**	**1.0**	**4.3**	**0.4**	-	-	-
1155	iso-pulegol	-	0.2	-	-	-	-
1165	**borneol**	**0.8**	-	**3.2**	**2.0**	**1.8**	**0.2**
1167	umbellulone	-	-	-	0.4	-	-
1172	cis-pinocamphone	0.1	-	-	-		t
1174	**terpinen-4-ol**	**1.9**	**13.5**	**9.8**	**0.7**	**0.6**	**0.1**
1179	p-cymen-8-ol	t	-	0.1	0.1	t	0.1
1186	α-terpineol	0.2	0.5	0.5	0.3	0.2	0.5
1193	4Z-decanal	0.2	-	-	-	-	-
1195	cis-piperitol	-	0.2	0.1	0.4	t	-
1195	myrtenol	-	-	-	-	-	t
1195	methyl chavicol	-	0.2	-	-	-	-
1207	trans-piperitol	-	0.4	0.5	0.5	0.1	-
1215	trans-carveol	-	-	-	-	-	t
1218	endo-fenchyl acetate	-	-	-	0.1	0.1	0.1
1223	**citronellol**	**1.5**	**1.4**	**0.3**	-	**t**	-
1232	thymol, methyl ether	-	-	0.1	t	-	0.1
1235	trans-chrysanthenyl acetate	0.5	-	-	-	1.1	-
1239	carvone	-	-	-	t	-	-
1241	carvacrol, methyl ether		-	-	-	-	t
1247	C10-OH, 41,109,119,152	0.6	-	-	-	-	-
1249	piperitone	-	-	0.2	0.4	-	-
1254	**linalyl acetate**	-	-	**t**	**0.2**	**0.1**	-
1255	4Z-decenol	0.2	-	-	-	-	-
1257	**methyl citronellate**	-	-	**0.2**	**t**	**0.1**	-
1274	pregeijerene B	0.5	1.0	0.9	0.3	0.9	-
1275	isopulegol acetate	-	-	-	-	0.1	-
1287	**bornyl acetate**	**0.9**	**0.1**	**10.7**	**44.2**	**34.8**	**1.1**
1289	thymol	0.1	-	0.3	-	-	-
1298	carvacrol	-	-	-	0.1	0.2	-
1312	citronellic acid	0.1	0.2	0.2	0.1	0.1	-
1346	**α-terpinyl acetate**	-	-	**0.2**	**0.6**	**0.1**	-
1403	methyl eugenol	t	0.3	-	-	-	-
1448	cis-muurola-3,5-diene	-	-	0.3	-	-	0.3
1417	(E)-caryophyllene	t	-	-	0.4	t	0.2

RI	Component	Salt	Zan	MC	MO	MM	Jal
1451	trans-muurola-3,5-diene	t	-	-	-	-	t
1452	α-humulene	-	-	-	-	-	0.3
1461	cis-cadina-1(6),4-diene	-	-	-	-	-	0.3
1465	cis-muurola-4(14),5-diene	-	-	-	-	-	0.5
1475	trans-cadina-1(6),4-diene	-	-	0.2	-	-	t
1480	germacrene D	-	-	-	-	-	1.1
1493	trans-muurola-4(14),5-diene	t	-	0.7	-	-	-
1493	epi-cubebol	-	-	0.4	-	-	-
1495	epi-cubebene	-	-	-	-	-	t
1500	α-muurolene	-	-	-	-	-	0.1
1501	epi-zonarene	-	-	-	-	-	0.1
1513	γ-cadinene	-	-	-	-	-	0.6
1513	cubebol	-	-	0.6	-	-	-
1521	trans-calamenene	-	-	-	-	-	0.1
1522	δ-cadinene	-	-	0.3	-	-	0.9
1528	zonarene	-	-	0.2	-	-	-
1531	cis-calamenene	-	-	t	-	-	-
1537	α-cadinene	-	-	-	-	-	0.1
1544	α-calacorene	-	-	-	-	-	0.1
1548	**elemol**	**1.4**	**2.5**	**5.7**	**2.4**	**5.4**	**-**
1559	germacrene B	-	-	-	0.1	0.2	-
1574	germacrene-D-4-ol	-	-	-	-	-	0.1
1582	caryophyllene oxide	-	-	-	0.1	-	t
1600	cedrol	-	-	t	0.2	-	-
1608	humulene epoxide II	-	-	-	-	-	t
1608	$C_{15}OH$, 43,109,119,220	0.1	-	-	0.5	0.6	-
1627	1-epi-cubenol	0.1	-	0.7	-	-	-
1630	γ-eudesmol	0.2	0.4	1.3	0.5	1.0	-
1638	epi-α-cadinol	-	-	-	-	-	1.1
1638	epi-α-muurolol	-	-	-	-	-	1.0
1644	α-muurolol	-	-	-	-	-	0.2
1649	β-eudesmol	0.3	0.4	2.6	0.5	0.9	-
1652	α-eudesmol	0.3	0.5	1.9	0.5	1.2	-
1652	α-cadinol	-	-	-	-	-	1.5
1670	bulnesol	0.1	0.1	0.4	0.3	0.5	0.1
1688	shyobunol	-	-	0.2	-	-	-
1746	8-α-11-elemodiol	0.3	0.2	0.2	-	0.2	-
1792	8-α-acetoxyelemol	0.3	0.7	2.0	0.5	1.2	-
1958	iso-pimara-8(14),15-diene	-	0.6	t	-	-	t
1988	**manoyl oxide**	**1.5**	**11.7**	**6.3**	**0.2**	**1.0**	**0.2**

RI	Component	Salt	Zan	MC	MO	MM	Jal
1987	iso-pimara-7,15-diene	-	-	-	0.2	0.5	-
2009	epi-13-manoyl oxide	-	0.1	t	-	-	-
2055	**abietatriene**	**0.3**	**3.4**	**3.1**	**0.3**	**1.1**	**0.3**
2087	abietadiene	-	0.3	t	t	0.5	0.1
2105	iso-abienol	0.3	-	1.4	-	0.1	-
2256	methyl sandaracopimarate	1.5	-	-	-	-	-
2282	sempervirol	0.4	0.1	0.1	-	-	-
2298	4-epi-abietal	-	-	0.1	-	t	t
2314	trans-totarol	0.2	t	0.6	0.1	0.7	0.6
2331	trans-ferruginol	0.1	t	0.1	-	t	t
	Number of cpds.	60	52	69	59	63	71

VARIATION IN nrDNA AND cpDNA OF *JUNIPERUS CALIFORNICA, J. GRANDIS, J. OCCIDENTALIS* AND *J. OSTEOSPERMA* (CUPRESSACEAE)

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ABSTRACT

Single Nucleotide Polymorphisms (SNPs) of nrDNA and cpDNA (petN-psbM, trnD-T, trnL-F, trnS-G) of *Juniperus grandis* were examined from throughout its range. All the sequences showed *J. californica* to be quite distinct. 27 SNPs from nrDNA displayed mosaic variation among *J. grandis-occidentalis-osteosperma* individuals. Four SNPs from petN-psbM showed the Yolla Bolly plants with no differences from *J. occidentalis* (Sisters, OR), supporting the terpene data that the Yolla Bolly Mtns. putative *J. grandis* population is a form of *J. occidentalis*. No systematic differences were found in petN-psbM between *J. grandis* and *J. osteosperma*. 2 SNPs from trnD-trnT separated *J. occidentalis* from *J. osteosperma-J. grandis*. Analysis of trnL-F gave no SNPs of systematic use. *Juniperus grandis* (Meyers, CA) was separated from *J. occidentalis* by 2 SNPs from trnS-trnG and from *J. grandis* (Big Bear)-*J. osteosperma* by 2 SNPs. These data concur with the terpene data in showing the divergence of *J. grandis* from the central High Sierra from the disjunct San Bernardino Mtns. (Big Bear), *J. grandis* population. None of the five regions sequenced could consistently separate *J. grandis* (Big Bear) from *J. osteosperma*. *Phytologia 92(2): 266-276 (August 2, 2010).*

KEY WORDS: *Juniperus grandis (= J. occidentalis* var. *australis), J. californica, J. occidentalis, J. osteosperma,* Cupressaceae, nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG, SNPs, geographic variation.

The western junipers consist of 4 species: *Juniperus grandis* R. P. Adams (= *J. occidentalis var. australis* (Vasek) A. & N. Holmgren), *J. californica* Carr., *J. occidentalis* Hook. and *J. osteosperma* (Torr.) Little (Adams, 2008). Adams and Kauffmann (2010, this issue), using leaf terpenoid data, found that the oil of the disjunct Yolla Bolly Mtns. (nw CA) population, often included in *J. grandis*, was slightly more similar to *J. occidentalis* than any population of *J. grandis* (Fig. 1). In
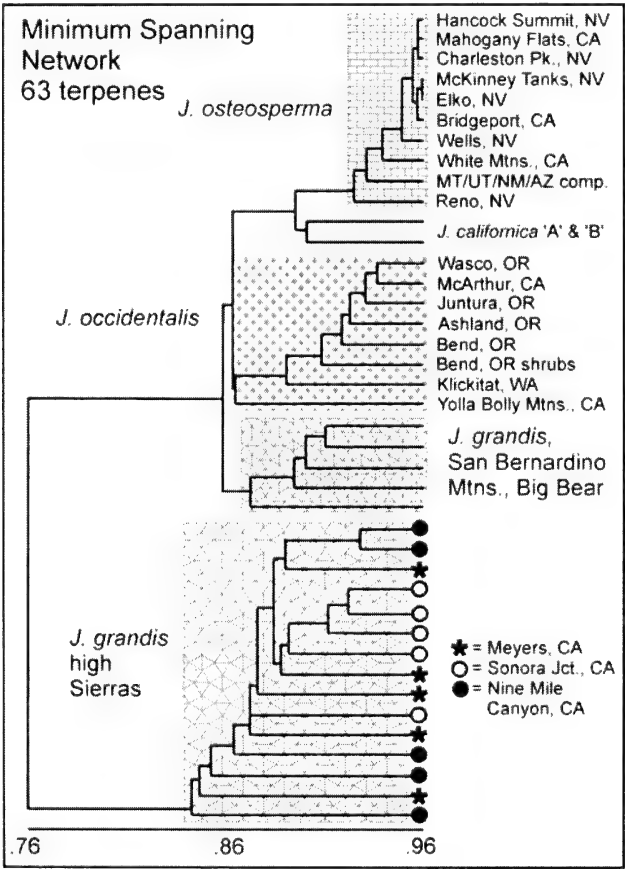

Figure 1. Minimum spanning network based on 63 terpenoids (from Adams and Kaufmann, 2010).

addition, they found that the oil of *J. grandis* from the High Sierras was quite different from *J. grandis* from the disjunct San Bernardino Mts. (Fig. 1). In fact, the oils of *J. grandis* from the San Bernardino Mts. were most similar to *J. occidentalis* (Fig. 4).

Juniperus grandis has an interesting distribution (Fig. 2) with populations in the high Sierras and the San Bernardino Mts. (Fig. 2),
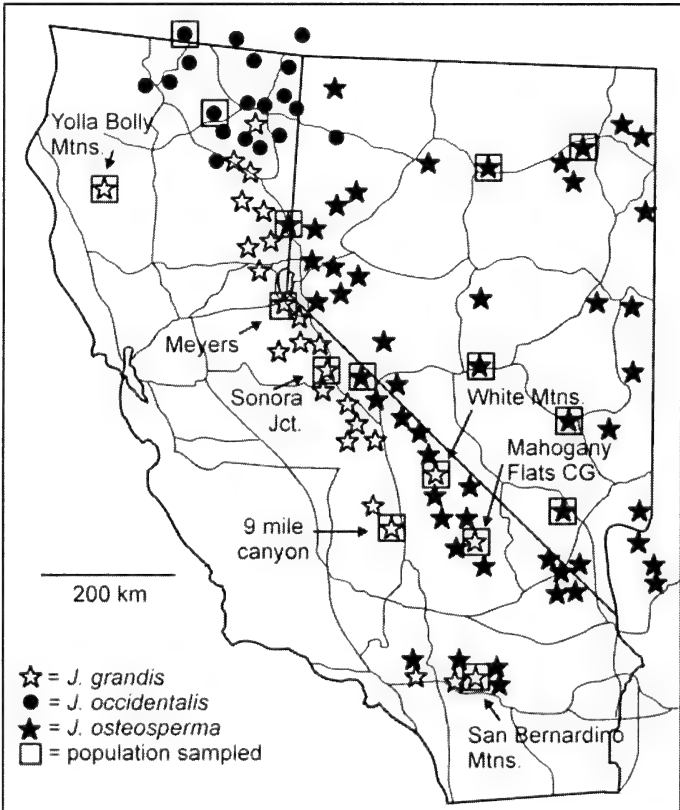
Figure 2. Distribution of *J. grandis* showing populations sampled. Partial distributions of *J. occidentalis* and *J. osteosperma* are also mapped in this region.

and, according to Vasek (1966), with putative outlying populations in the Yolla Bolly Mtns., White Mtns., and Panamint Range (see Mahogany Flats CG, Fig. 2). However, the oils from putative *J. grandis* from the White Mtns. and Panimint Range (Mahogany Flats) appeared to be typical *J. osteosperma* (Fig. 1).

The purpose of this study was to examine DNA sequences from the same trees of *J. grandis* sampled for leaf oils (Adams and Kauffmann, 2010). nrDNA has been widely used in *Juniperus* studies along with petN-psbM (Adams, 2009; Adams et al., 2009; Adams et al., 2010a, b, c, d; e Terry et al., 2000). Recently, Mao et al. (2010) have reported on the utility of several cpDNA regions for systematics of *Juniperus*. Three of their most promising cp regions were trnD^{GUC}-trnT^{GGU}, trnL^{UAA}-trnF^{GAA} and trnS^{GCU}-trnG^{UCC}. These 3 cp regions were also utilized in this study.

MATERIALS AND METHODS

Plant material J. californica, 'A', Adams 10154-10156, Victorville, CA, Adams 85-8697, 13 km n of Amboy/Kelso exit on I40, on road to Kelso at Granite Pass, 34° 48.41N, 115° 36.54'W, 1280 m; *J. californica,* 'B', *Adams 8698-99,* 27 km se of SE of Yucca, AZ on Alamo Road, 34° 44.91N, 113° 58.19'W, 920 m;
J. grandis, Adams 11963-11967, Jct. US 50 & CA 89, 38° 51.086N, 120° 01.244'W, 1937 m, Meyers, CA, Adams 11968-11972, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289'N, 111° 35.598'W, 2585 m, Tuolumne Co., CA, *Adams 11984-11988,* Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003'N, 118° 02.078'W, 2059 m, Tulare Co., CA, *Adams 11989-11993,* 5km n Big Bear City on CA 18, 34° 17.533'N, 116° 49.153'W, 2053 m, San Bernardino Co., CA;
J. occidentalis, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392'N, 120° 41.207'W, 170 m, Klickitat Co., WA, *Adams 11943-11945,* 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR, 44° 53.676'N, 120° 56.131'W, 951 m, Wasco Co., OR, *Adams 11946-11948,* 3 km sw of Bend, OR, on OR 372, 44° 02.390'N, 121° 20.054'W, 1132 m, Deschutes Co., OR, *Adams 11949-11951,* 32 km e of Bend, OR on OR20, shrubs, 0.5 - 1 m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, Deschutes Co., OR, *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR66, 42° 08.044'N, 122°

34.130'W, 701 m, Jackson Co., OR, *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m, Lassen Co., CA, *Adams 11995-11998 (Kaufmann A1-A3, B1)*, Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA;

J. osteosperma, Adams 10272-10276, on NV157, Charleston Mtns. 36° 16.246'N, 115° 32.604'W, 1795 m, Clark Co., NV, *Adams 11122-11124*, Hancock Summit, mile 38 on US375, 37° 26.404'N, 115° 22.703'W, 1675 m, Lincoln Co. NV, *Adams 11125-11127*, McKinney Tanks Summit on US6, 38° 07.005'N, 116° 54.103'W, 1933 m, Nye Co., NV. *Adams 11134-36*, 8 km s of Bridgeport, on US395, 38° 12.639'N, 119° 13.846'W, 2004 m, Mono Co., CA; *Adams 11141-11143*, 13 km w of Elko, on I80, 40° 45.598'N, 115° 55.942'W, 1535 m, Elko Co., NV, *Adams 11144-11146*, 8 km e of Wells, on I80, 41° 06.533'N, 114° 51.441'W, 1876 m, Elko Co., NV, *Adams 11960-11962*, 56 km n of Reno, NV, 39° 54.458'N, 120° 00.322'W, 1383 m, Lassen Co., CA, *Adams 11973-1977*, 10 km n of CA 168 on White Mtn. Rd., 37° 20.143'N, 118° 11.346'W, 2607 m, Inyo Co., CA, *Adams 11978-11982*, 36° 13.783'N, 117° 04.102'W, 2477 m, Inyo Co., CA. Voucher specimens are deposited at the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The primers for trnD-trnT, trnL-trnF and trnS-trnG regions:
trnD for ACC AAT TGA ACT ACA ATC CC   cf. Grivet et al., 2001
trnT rev CTA CCA CTG AGT TAA AAG GG

trnL for CGA AAT CGG TAG ACG CTA CG   cf. Taberlet et al. 1991
trnF rev ATT TGA ACT GGT GAC ACG AG

trnS for GCC GCT TTA GTC CAC TCA GC   cf. Shaw et al., 2005
trnG rev GAA CGA ATC ACA CTT TTA CCA C

     The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

RESULTS AND DISCUSSION

     Sequencing nrDNA for 30 individuals revealed 37 mutational events with 10 single mutations (occurred only once among the 30 individuals) and 27 mutations that occurred more than once, implying systematic information might be present. A minimum spanning network based on these 27 SNPs is shown in Figure 3. *Juniperus californica* is quite separated by at least 20 SNPs from the other taxa. However, none of the other three species are separated.   There are neither specific nor geographic differences among *J. grandis, J. occidentalis* nor *J. osteosperma*. The pattern is completely mosaic. Clearly, nrDNA data is quite conserved among these species. This is similar to the situation in *Cupressus* from the western hemisphere (now *Hesperocyparis*, see Adams, Bartel and Price, 2009), where Little (2006) reported no variation in nrDNA among 11 *Cupressus* species!

     Sequencing petN-psbM resulted in 4 SNPs from 853 - 855 bp of data. The petN-psbM SNPs show *J. californica* to be distinct, with 1 SNP separating *J. occidentalis* (Sisters, OR and Yolla Bolly, CA) from *J. grandis* (12 individuals from 4 populations) and *J. osteosperma* (3 trees, Salt Lake City, UT) (Figure 4, left).
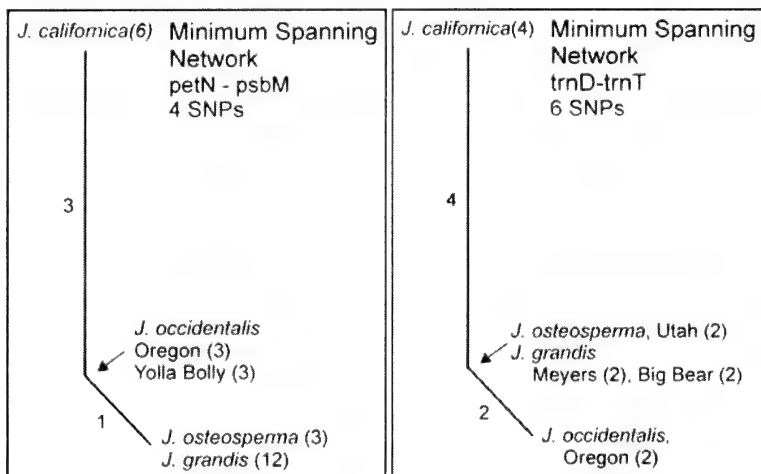
Figure 3. Minimum spanning network based on 27 SNPs from nrDNA sequencing (1250 - 1273 bp).

Sequencing the trnD-trnT region gave 6 systematically useful SNPs from 685 - 686 bp of data. Again, *J. californica* is well separated by 4 SNPs (Figure 4, right). No systematic variation was found among *J. osteosperma* (2), *J. grandis* (Meyers, 2; Big Bear 2) nor among the *J. occidentalis* (Sisters, OR, 2). No SNPs were found that separated *J. osteosperma* from *J. grandis*, or the Meyers and Big Bear *J. grandis* populations.

Preliminary sequencing of the trnL-trnF region (701-702 bp) for *J. grandis* (2), *J. occidentalis* (2) and *J. osteosperma* (2) yielded only one

Figure 4. Minimum spanning networks. Left: petN-psbM. Right: trnD-T.

single point insertion in one individual, so sequencing of additional individuals was not continued.

Sequencing trnS-trnG resulted in 815 - 818 bp of data with 9 SNPs. Analysis of these SNPs (Fig. 5) shows *J. californica* well resolved (5 SNPs) with *J. occidentalis*, *J. grandis* (Meyers, CA) and *J. grandis* (Big Bear) - *J. osteosperma* separated by 2 SNPs each. Interestingly, *J. grandis* (Big Bear) - *J. osteosperma* is separated by 4 SNPs from *J. occidentalis* (Fig. 5).

Overall, only trnS-trnG separated *J. grandis* into two groups (Meyers and Big Bear) as found in the terpene data (Adams and Kaufmann, 2010). The Big Bear *J. grandis* appeared to be more like *J. occidentalis* in its terpenes and more like *J. osteosperma* in its SNPs.

Vasek (1966) reported finding *J. grandis* (*J. occidentalis* var. *australis*) individuals with affinities to *J. osteosperma* in the San Bernardino Mtns. as well as what he considered to be typical *J. grandis* and *J. osteosperma*. In the High Sierras, *J. grandis* grows on very xeric exposed granite domes, but its habitat in Big Bear (San Bernardino Mtns.) is on alluvial deposits. It may be found on exposed granite at higher sites
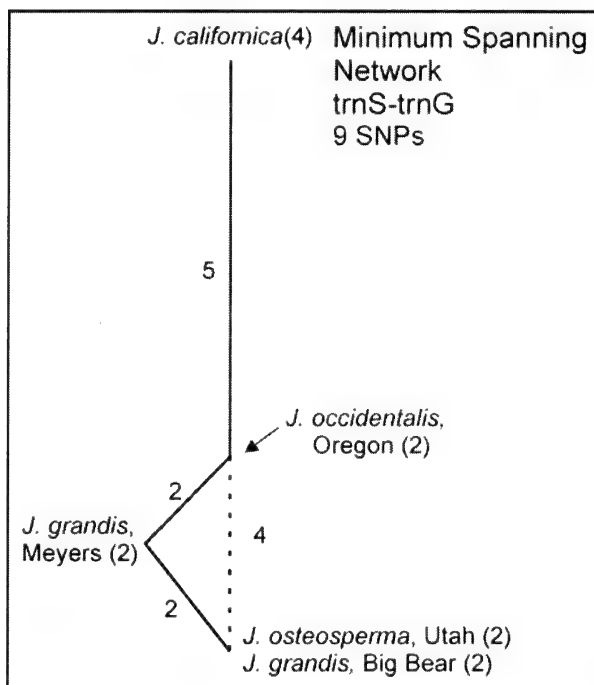
Figure 5. Minimum spanning network based on trnS-trnG SNPs.

and additional collections are being made from the San Bernardino Mtns. to further investigate the interactions between *J. grandis* and *J. osteosperma*. At present, the Big Bear population of *J. grandis* does not appear to be typical of *J. grandis* from the High Sierras in its oil or DNA. Additional DNA markers are being screened for use in resolving this taxonomic problem.

The petN-psbM SNPs data gives support to the terpene data that the Yolla Bolly population is a variant of *J. occidentalis*. Additional research is needed to clarify its taxonomic status.

ACKNOWLEDGEMENTS

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Seed Dispersal: harvester ant carrying female cone of *Juniperus arizonica* to nest

Phytologia

Contents

Cover Photo. Harvester ant carrying a female cone of *Juniperus arizonica* to its nest. Photo by David Thornburg.

MEXICAN SPECIES OF THE GENUS *BARTLETTINA* (ASTERACEAE: EUPATORIEAE), AND ERECTION OF THREE NEW SPECIES

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ABSTRACT

The Mexican species of *Bartlettina* are revised. Three new species from the state of Oaxaca, **B. juxtlahuaca, B. serboana** and **B. yaharana**, are proposed. As currently conceived, Mexico contains 21 species of *Bartlettina*, most of these occurring in southern portions of the country. A key to the taxa is provided, along with maps showing their distribution, all of this in the format of my previously published Eupatorieae of Mexico (Phytologia Memoirs, Vol. 11, 1997). *Phytologia 92(3): 279-303 (December 1, 2010).*

KEY WORDS: Asteraceae, Eupatorieae, *Bartlettina*, Mexico, Oaxaca

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BARTLETTINA King & H. Rob.
Neobartlettina King & H. Rob. (not *Neobartlettina* Schlecht.)

Robust suffruticose herbs, shrubs, or small trees, 1-7 m high. Stems densely hirsute-velvety to glabrous, pithy or rarely hollow, the nodes sometimes flattened. Leaves opposite; petioles well-developed; blades pinnately veined to 3-9 nervate from the base, glandular-punctate or not. Heads usually numerous in broad rounded or pyramidal corymbose-panicles, less often 1-15 in lax corymbs. Involucres (2)3-7 seriate, gradate, or rarely not, usually closely appressed and persistent. Receptacles convex or plane, pubescent or glabrous, epaleate, often alveolate. Corollas lavender (rarely white), tubular or funnelform, glabrous, or the lobes pubescent. Anther appendages mostly well-developed. Style branches slender, the apices scarcely enlarged.

Achenes 4-5 ribbed, the pappus of 30-50 persistent bristles. Base chromosome number, x = 16 (or perhaps both x =10 and 16).

Type species, *Eupatorium tuerckheimii* Klatt

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In North America, a largely natural group of 25-30 species (King and Robinson 1987), mostly confined to montane cloud forests. In South America, however, the complex appears to grade into yet and other poorly known groupings such as *Hebeclinium* and *Guayania* (Turner, unpubl.). King and Robinson (1971a) relate *Bartlettina* to *Decachaeta*, both of which possess base chromosome numbers of x = 16 and, usually, pubescent receptacles. Indeed, a case might be made for the expansion of *Decachaeta* to include the *Bartlettina* group, especially since *Decachaeta perornata,* with a haploid chromosome number of n = 16, appears to link the two taxa, possessing most of the attributes of *Bartlettina*, but having the reduced anther appendages of *Decachaeta*. The *Hebeclinium* and *Peteravenia* groups may also

possess pubescent receptacles but both possess base numbers of $x = 10$.
Bartlettina, as presently conceived (including the Critonioid elements
projected into the genus by Whittemore, 1985), is clearly polyphyletic.
I have keyed the monotypic genus *Amolina*, also with a base of $x = 10$,
in the following accounting because it superficially resembles a species
of *Bartlettina*. The taxonomic lines between these various taxa are in
much need of clarification.

KEY TO SPECIES

1. Involucres 4-9 mm high...**(4)**
1. Involucres 10-20 mm high...**(2)**

2. Involucral bracts herbaceous, pubescent............**B. constipatiflora**
2. Involucral bracts scarious, glabrous or nearly so....................**(3)**

3. Leaves broadly ovate to deltoid; heads ca 2 cm high.....**B. lanicaulis**
3. Leaves lanceolate, widest near the middle; heads ca 1.2 cm high....
....**B. platyphylla**

4(1) Involucral bracts subequal; leaves prominently atomiferous-
        glandular beneath, and very reticulate-veiny; Hid
        ....**B. ehrenbergii**
4.   Involucral bracts graduate; leaves not as above; widespread.....**(5)**

5. Stems, capitulescence and foliage variously puberulent, hirsute or
   glabrous but not as described below....................................**(9)**
5. Stems, capitulescence and foliage densely and conspicuously
   brownish- or purplish-velutinous.......................................**(6)**

6. Achenes 3.5-4.5 mm long; heads turbinate with 15-25 florets; Cps
   ....**Amolina heydeana**
6. Achenes 1.5-2.5 mm long; heads campanulate with 50-200 florets.**(7)**

7. Leaves variously ovate to subcordate or flabellate, not equally
   tapering at both ends; petioles 1 cm long or more..........**B. sordida**
7. Leaves elliptical, equally tapering at both ends; petioles 0.5-1.0 cm
   long; Oax..**(8)**

8. Petioles 0.5-1.0 cm long; Oax…..………………..…...….**B. calderonii**
8. Petioles 1.0-2.5 cm long; Tam, San, Hid……..……..….**B. xalapana**

9(**5**). Heads 8-12 mm wide, arranged in small, 5-18-headed, terminal
    corymbs; Hid, Pue, Ver………..……………..…**B. karwinskiana**
9.   Heads 4-10 mm wide, arranged in numerous-headed usually
    broad, rounded or pyramidal corymbs…………..………….**(10)**

10. Leaf blades variously elliptical, widest at or near the middle, 3-6
    times longer than wide……………………………………………**(15)**
10. Leaf blades ovate to deltoid, widest below the middle, 1.5-2.5
    times longer than wide……………………………………………**(11)**

11. Leaf blades acute or rarely obtuse at the base; capitulescence a
    large, rounded, numerous-headed, compact, corymbose-panicle 2-3
    times as wide as long; Cps………………………**B. pansamalensis**
11. Leaf blades cordate, truncate to obtuse at the base (very rarely
    acute); capitulescence various, about as long as wide or longer..**(12)**

12. Innermost involucral bracts densely puberulent throughout, mostly
    acute at the apex; mature leaves readily seen to be pellucid-punctate;
    florets conspicuously pubescent on the lobes and extending onto the
    throat…………………………………………………..**B. breedlovei**
12. Innermost involucral bracts merely ciliate or very sparsely
    puberulent, mostly obtuse at the apex; mature leaves
    inconspicuously pellucid-punctate; florets (5)10-30,
    inconspicuously pubescent, the hairs confined to the lobes……..**(13)**

13. Florets 50 or more per head; leaves 15-30 cm wide…...**B. yaharana**
13. Florets 5-30 per head; leaves 5-12 cm wide……………...……..**(14)**

14. Florets 5-13 per head; blades of leaf often with lateral flanges
    ……………………………………………………………**B. ornata**
14. Florets 15-30 per head; blades without flanges………….**B. oresbia**

15(**10**). Corollas, upper part, densely puberulent; branches of
    capitulescence glabrous or nearly so……………………….**(18)**
15.   Corollas, upper part, glabrous; branches of capitulescence
    rather evenly puberulent………………………………………**(16)**

16. Receptacles pubescent..................................**B. tamaulipana**
16. Receptacles glabrous..**(17)**

17. Leaf blades 7-9 cm wide, widest at the middle; petioles 5-6 cm
    long; Oax...**B. serboana**
17. Leaf blades 3-4 cm wide, widest off-center; petioles 3-4 cm long;
    Cps...**B. pinabetensis**

18(**15**). Heads 20-40 flowered; body of achene variously sparsely
       pubescent or setiferous; receptacle 1.0-1.5 mm wide, glabrous
       or prominently pubescent..**(20)**
18.   Heads 50-150 flowered; body of achene glabrous;
       receptacle 1.5-2.5 mm wide, pubescent..........................**(19)**

19. Leaves markedly evenly serrate; heads with ultimate peduncles
    1-5 mm long...**B. juxtlahuaca**
19. Leaves entire to undulate, not serrate; ultimate peduncles 5-10 mm
    long..**B. tuerckheimii**

20. Heads white; peduncles mostly ebracteate and 6 mm long or longer;
    leaves narrow, undulate to nearly entire, without well-developed
    serrations; petioles 1-3 cm long..............................**B. cronquistii**
20. Heads lavender to purple; peduncles mostly 6 mm long or less;
    leaves variously serrate, usually with well-developed marginal
    venations; petioles 0.5-1.0 cm long..............................**(21)**

21. Petioles, and often the mid-ribs, puberulent; receptacle pubescent
    ....**B. hintonii**
21. Petioles and mid-ribs glabrous; receptacle glabrous..**B. macdougalii**

BARTLETTINA BREEDLOVEI King & H. Rob., Phytologia 28:
    286. 1974. **Map 1**
    Eupatorium breedlovei (King & H. Rob.) B.L. Turner (1989); not
    E. breedlovei (King & H. Rob.) B.L. Turner (1987)
    Eupatorium tenejapanum B.L. Turner

    Cps and adjacent Guatemala, evergreen montane cloud forests,
1300-3000 m; Dec-Feb.

Robust, suffruticose herbs or shrubs 1-3 m high; superficially very similar to *B. oresbia* but distinguished by its longer involucral bracts (the inner series of which are densely puberulent throughout with mostly acute apices), conspicuously pellucid-punctate leaves, and more numerous florets per head (30-50 vs 10-30); chromosome number, $n =$ 16 pairs.

Although *B. breedlovei* and *B. oresbia* are sympatric in Cps it appears that they occupy largely different habitats: the former in cloud forest at mostly higher elevations (2000-3000 m), the latter in lower cloud forests (1000-2200 m).

BARTLETTINA CALDERONII (B.L. Turner) B.L. Turner, Phytologia 82: 385. 1997. **Map 1**
Eupatorium calderonii B.L. Turner

Known only from north-central Oax, 800-1600 m; Mar-Apr
Perennial suffruticose herb or subshrub to 2-3 m high; very similar to *B. sordida* but the leaves elliptic, about equally tapered at both ends, the petioles 1 cm long or less and the heads only ca 30-flowered, the achenes decidedly hispid above.

A poorly known taxon that might ultimately prove to be but a form of *B. sordida*; the characters enumerated above which distinguish between them suggest a distinct species.

BARTLETTINA CONSTIPATIFLORA (Klatt) King & H. Rob., Phytologia 22:160. 1971. **Map 2**
Eupatorium constipatiflorum Klatt
Neobartlettina constipatiflora (Klatt) King & H. Rob.

Known only from central Oax, cloud forests, 2500-2900 m; Jan-Feb.
Shrubs 2-3 m high; leaves mostly 15-20 cm long, 6-9 cm wide; petioles 3-5 cm long, densely purplish-puberulent; blades broadly ovate to somewhat trullate, penninervate, sparsely pubescent and atomiferous-glandular beneath, the margins minutely serrulate; heads relatively large, 5-20 in stiffly-arranged terminal corymbs, the ultimate peduncles mostly 1-3 cm long; involucres campanulate, 12-13 mm high, 15-20 mm wide, the bracts 5-6 seriate, strongly gradate, the

middle and outer series ovate, herbaceous; corollas numerous (100+), the lobes puberulous; achenes ca 3 mm long, glabrous, the pappus of 50-60 white bristles ca 6 mm long.

Type material (largely fragmentary) of this taxon shows the heads to be congested; recent collections from or near the type locality show the capitulescence to be a stiffly-branching corymb, the ultimate peduncles 1-3 cm long.

BARTLETTINA CRONQUISTII King & H. Rob., Phytologia 38:108. 1977. **Map 2**
Eupatorium cronquistii (King & H. Rob.) B.L. Turner

Montane regions along the Pacific slopes, se Jal, Mic? Gue?,and Oax, 1600-1800 m; Nov-Mar.

Erect glabrous shrubs to ca 2 m high; vegetatively very similar to *B. tuerckheimii* but the heads white, turbinate (3-5 mm wide), the involucres with fewer bracts and only 18-22 florets.

Related to, but clearly distinct from, *B. tuerckheimii* and *B. macdougalii*, the latter from the Gulf slopes of Ver and Oax. Though not reported for Jal by McVaugh (1984), recent collections of what appear to be *B. cronquistii* have been obtained from Sierra Manantlan (*Villalobas 192*, WIS).

BARTLETTINA EHRENBERGII (Hemsl.) King & H. Rob., Phytologia 22: 160. 1971. **Map 2**
Bartlettina macrocephala (Benth.) King & H. Rob.
Eupatorium ehrenbergii Hemsl.

Known only from the vicinity of Pachuca and Zimapan, Hid, where it mostly occurs on steep slopes and canyon walls, ca 1000 m; Feb-Mar.

Suffruticose perennial herb or shrublet to 1.4 m high; stems densely grey-hirsute, with age corky; leaves opposite, 8-15 cm long, 3-6 cm wide; petioles 1.5-3.0 cm long; blades ovate to ovate-elliptic, densely grey-pilose, abundantly nervose and atomifeous-glandular beneath, the margins dentate; heads 3-13, pale lavender, in terminal corymbose-panicles somewhat wider than high; involucres 3-4 seriate, the bracts subequal, 6-8 mm long, 1.0-1.3 mm wide, pubescent; receptacle

convex, densely pubescent; florets 50-100 or more, the corolla lobes pubescent; achenes ca 2.5 mm long, densely hispidulous, the pappus of 30-40 bristles 3-5 mm long, not at all expanded at the apices.

A very distinct species not easily confused with another in the *Bartlettina* complex.

BARTLETTINA HINTONII King & H. Rob., Phytologia 31: 62. 1975. **Map 3**
Eupatorium hintonii (King & H. Rob.) B.L. Turner

Known only from two collections; the type, Distr. Galeana, Piedra Ancha - Tres Cruces, Gue, in oak forests, 2500 m and from Tlacotepec, ca 10 km NE of Atoyac, 2580 m in pine-oak forests; Dec.

Shrubs 1.5-5.0 m high; superficially similar to *B. tuerckheimii* but the leaves closely serratulate, pubescent beneath along the veins and the heads pale lavender or lilac, arranged in neatly rounded terminal corymbs about as wide as long; chromosome number, n = ca 16 pairs.

BARTLETTINA JUXTLAHUACA B.L. Turner, **sp. nov**. **Fig. 1., Map 3**

Bartlettina tuerckheimii (Klatt) King & H. Rob. Similis sed differt laminas foliorum valde aequaliter serratis (vs. integris vel paene integris) apicibus parum attenuates (vs. valde attenuates) et pedunculis ultimis plerumque brevioribus (1-5 mm longis vs. 6-10 mm).

Shrubs 3-4 m high. **Stems** purplish, glabrous, or nearly so. **Leaves** 10-18 cm long, 3.0-4.5 cm wide; petioles 5-10 mm long; blades lanceolate, grading into the petioles, their margins rather evenly and markedly serrate. **Heads** numerous in congested terminal corymbs, their ultimate peduncles 1-5 mm long. **Involucral bracts** 4-5 seriate, imbricate, ovate (outer) to linear-lanceolate (inner), pubescent. **Receptacle** convex, 2-3 mm across, pubescent. **Florets** 30-40; corollas lavender, the lobes pubescent. **Achenes** glabrous, ca 3.5 mm long; pappus of 40-50 persistent white bristles 4-5 mm long.

TYPE: **MEXICO. OAXACA: Distr. Juxtlahuaca, Mpio. San Martin Peres,** "1 km de la desviacion para el pobldo La Escopeta, carretera Santiago Juxtlahuaca-San Martin Peres," pine-oak forests, ca 2625 m, 16 Dec 1996, *J.I. Calzada 20854* (Holotype, MEXU; isotype, TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. OAXACA: Mpio. San Martin Peres,** "1 km de la desvacion del poblado de Escopeta, km 24 carretera San Sebastion Tecomoxtlahuaca, ca 2655 m, 29 Nov 1994, *Calzada 19562* (TEX); **Mpio. Santiago Juxtlahuaca**, "Entrada al pobla de El Manzanal," ca 2255 m, 16 Dec 1995, *Calzada 20664* (TEX).

Bartlettina juxtlahuaca superficially resembles *B. cronquistii* of the Pacific slopes, but is readily distinguished from the latter by its shorter petiolate, uniformly serrate, leaves.

The species is named for the Distr. Juxtlahuaca, whence the type.

BARTLETTINA KARWINSKIANA (DC.) King & H. Rob.,
Phytologia 21: 161. 1971. **Map 4**
Eupatorium karwinskianum DC.
Jaumea tenuifolia Klatt

Hid, Ver, Pue and Oax in montane cloud forest along barrancas from 1500-2000 m; Jan-May.

Robust perennial, suffruticose herb, or small shrub, 0.6-2.0 m high; stems puberulent to glabrate; leaves opposite, 4-14 cm long, 2-5 cm wide; petioles 1-5 cm long; blades ovate to rhombic-ovate, penninervate to 3-nervate from above the base, glandular-punctate beneath; heads lavender (5)10-18, arranged in relatively loose, terminal, corymbs; involucres 5-6 seriate, imbricate, 6-8 mm long, 8-10 mm wide; florets 60-100 per head, the corollas with pubescent lobes; achenes 1.5-2.0 mm long, glabrous or nearly so, the pappus of 40-50 bristles 4-6 mm long, not, or but slightly, dilated at the apex.

This species was excluded from Standley's Trees and Shrubs of Mexico, but recent collections indicate the plant to be, upon occasion, a shrub. It resembles *B. oresbia* in habit but possesses fewer, larger, heads with more numerous florets.

BARTLETTINA LANICAULIS (B.L. Rob.) B.L. Turner, Phytologia
    82: 386. 1997. **Map 4**
    Critonia lanicaulis (B.L. Rob.) King & H. Rob.
    Eupatorium lanicaule B.L. Rob.

Cps and adjacent Guatemala southwards, seasonal evergreen forests,
300-1200 m; Dec-Feb.

Slender suffruticose tall herbs or shrubs 1-5 m high; stems tawny-
hirsute, leaves opposite throughout, 10-28 cm long, 3-6 cm wide;
petioles 1-10 mm long; blades lanceolate to lanceolate-elliptical,
pinnately nervate, the margins serrate to serrulate; heads mostly 3-12 in
a terminal capitulescence, the ultimate peduncles 2-12 mm long;
involucres broadly campanulate, 12-16 mm high, 6-8 seriate, the bracts
markedly gradate with obtuse or rounded apices, the inner bracts
readily disarticulating with age; corollas white; achenes linear, 5-6 mm
long, glabrous, 4-5 ribbed, the pappus of 35-40 tawny bristles 7-8 mm
long.

This species, what with its large turbinate heads and markedly
gradate involucres, superficially resembles a member of the genus
Chromolaena but, as noted above, King and Robinson position it in
Critonia, whereas Whittemore (1985) would relegate it to the
Hebeclinium group of *Eupatorium*. The taxon clearly needs additional
study.

BARTLETTINA MACDOUGALII King & H. Rob., Phytologia 38:
    107. 1997. **Map 4**

Pue, Ver and Oax in montane cloud forests with tree ferns, Gulf
slopes, 1200-3000 m; Aug-Mar.

Suffruticose, perennial, glabrous herbs or shrubs, 1-3 m high; much
resembling *B. tuerckheimii* but differing by its broader leaves (mostly
4-8 cm wide vs 2-4 cm) and glabrous or very sparsely pubescent,
alveolate, receptacles; chromosome number, n = ca 16 pairs.

More extensive collections in the border areas of Oax and Cps may
show this taxon to be but varietally distinct from *B. tuerckheimii*.

BARTLETTINA ORESBIA (B.L. Rob.) King & H. Rob., Phytologia 22: 161. 1971. **Map 5**
 Bartlettina guatemalensis King & H. Rob.
 Bartlettina hylobia (B.L. Rob.) King & H. Rob.
 Bartlettina oresbioides (B.L. Rob.) King & H. Rob.
 Eupatorium hylobium B.L. Rob.
 Eupatorium oresbioides B.L. Rob.
 Eupatorium oresbium B.L. Rob..

Sin, Dur, Jal, Hid, Mex, Mor, Pue, Ver, Gue, Oax and Cps, Guatemala to El Salvador in pine-oak forests; 1200-2600 m; Dec-Apr.

Perennial suffruticose herbs, shrubs or small trees, 1-5 m high; stems puberulent to glabrate; leaves 10-20 cm long, 3-12 cm wide; petioles 3-7 cm long; blades broadly ovate, rhomboid, deltoid to narrowly ovate, penninervate, pubescent at first but soon glabrate, except for the hirsute venation, the margins serrate to crenate, weakly pellucid-punctate beneath; heads lavender, turbinate, numerous, in pyramidal or rounded corymbose panicles; involucres 3-4 seriate, unevenly imbricate, (4)5-7 mm high, the outer series subtended by 3-5 calyculate bracts; florets 15-30 per head, the corollas 4-6 mm long, the lobes sparsely pubescent to glabrate; achenes 1.6-2.2 mm long, glabrous, the pappus of 30-40 bristles, 4-6 mm long, the apices not dilated; chromosome number, *n* = 16 pairs.

This is an exceptionally widespread, variable species, especially in Cps and Guatemala where, perhaps, it hybridizes with other taxa. I can find no justification for the recognition of *B. hylobia* from Cps, the type of which scarcely differs from *B. oresbia* as I sense the group. *Bartlettina oresbia* might also be compared with *B. ornata*, the latter being distinguished by its smaller heads with fewer florets.

BARTLETTINA ORNATA (B.L. Rob.) King & H. Rob., Phytologia 22: 172. 1991. **Map 5**
 Eupatorium ornatum (King & H. Rob.) B.L. Turner

se Cps and Guatemala, moutane rain forests, 2000-3000 m; Nov-Apr.

Much resembling *B. oresbia* but readily distinguished by its smaller, nearly cylindrical involucres with fewer florets (5-13 per head vs. 14-25).

BARTLETTINA PANSAMALENSIS (B.L. Rob.) King & H. Rob., Phytologia 22: 161. 1971. **Map 5**
Eupatorium pansamalense (B.L. Rob.) B.L. Turner
Bartlettina ruae (Standley) King & H. Rob.
Eupatorium ruae Standley

Cps and Guatemala to Honduras; in montane cloud forest, 1500-2500 m; Feb-Apr.
Shrubs or small trees, 2-7 m high; stems terete, pithy or hollow; very similar to *B. oresbia* and *B. breedlovei* but the heads larger, campanulate, with more numerous florets per head (30-60 vs 10-30).
Bartlettia pansamalensis, B. oresbia and *B. breedlovei* are sympatric in Cps and perhaps hybridize in this region upon occasion, although I have seen no clear intermediates. However, occasional intermediates between the former two taxa have been seen in Guatemala, and they can be expected in Cps. I agree with King & Robinson (1974) in the synonymy of *E. ruae* with *B. pansamalensis*. Williams (1976) treated both of the latter as synonymous with *Eupatorium luxii*.

BARTLETTINA PINABETENSIS (B.L. Rob.) King. & H. Rob., Phytologia 22: 161. 1971. **Map 6**
Eupatorium pinabetense B.L. Rob.
Neobartlettia pinabetensis (B.L. Rob.) King & H. Rob.

Cps and adjacent Guatemala in montane cloud forests, 700-2850 m; Jan-Mar.
Erect shrubs or spindly trees, 2-6 m high; leaves 10-20 cm long, 2-4 cm wide; stems evenly brown-puberulent to glabrate, petioles 1-4 cm long; blades pinnately veined, linear-elliptical to elliptical, glabrous or nearly so, the margins closely serratulate, heads lavender-blue, numerous, arranged in broad, rounded, terminal corymbs, 15-30 cm across; involucres narrowly turbinate, 2-3 seriate, unequally gradate, 5-

7 mm long, the bracts relatively few (12-18); receptacles glabrous; florets 10-15 per head, the corollas 3.5-4.5 mm long, the lobes glabrous or glandular; achenes 1.9-2.1 mm long, glabrous, the pappus of 30-40 bristles, 3-5 mm long.

The narrow involucres with relatively few florets distinguish this species from related taxa. Vegetatively, it is similar to *B. hintonii* of Gue, but the latter has more numerous florets per head (20-30 vs 10-15) and its receptacles and corolla lobes are glabrous.

BARTLETTINA PLATYPHYLLA (B.L. Rob.) King & H. Rob., Phytologia 22: 161. 1971. **Map 6**
Eupatorium platyphyllum B.L. Rob.

Pue, Ver, Oax, Cps, and Guatemala southwards, cloud forests, 400-1200 m; Jan-Apr.

Shrub or small tree, 2-10 m high; stems densely sordid or tawny-puberulent; leaves 10-25 cm long, 5-15 cm wide; petioles 2-7 cm long; blades broadly ovate to rarely cordate, 3-nervate from the base, puberulous above and below, especially along the major veins, moderately pellucid-punctate beneath, the margins serrate to nearly entire; heads campanulate, white, numerous in pyramidal corymbose panicles 10-30 cm across; involucres 6-7 seriate, evenly imbricate, 10-18 mm high, the bracts ovate (outer series) to linear-oblong (inner series), chartaceous to marginally scarious, 3-8 costate, the apices rounded; florets 50-90 per head, the corollas 6-9 mm long, glabrous; achenes 2.0-2.5 mm long, glabrous or rarely glandular, the pappus of 50-65 bristles, 8-10 mm long.

A very distinct species of *Bartlettina* and not readily confused with another.

BARTLETTINA SERBOANA B.L. Turner, **sp. nov., Fig. 2, Map 7**

Oax, known only from Distrito Juchitan, montane forests, ca 1710 m, ; Dec.

Bartlettinae pinabetensi similis sed laminis foliorum late ellipticis majoribus (7-9 cm latis vs. 3-4 cm), petiolis longioribus (5-6 cm longis vs. 3-4 cm), et corollis albis (vs. lilacini-azureis) differt.

Resembling *Bartlettina pinabetensis* but the leaf blades broadly elliptic, larger (7-9 cm wide, vs 3-4 cm), petioles longer (5-6 cm long vs 3-4 cm) and the corollas white (vs lavender-blue)

Shrublet or suffruticose herbs to 2 m high. **Stems** pubescent with brown puberulent hairs. **Leaves** opposite, ca 22 cm long, 5-7 cm wide; petioles 5-6 cm long; blades elliptic, glabrous, widest at or near the middle, the margins weakly serrate. **Capitulescence** a numerous-headed, corymbose panicle, ca 20 cm wide, and as long, the ultimate peduncles 2-3 mm long. **Involucres** ca 4 mm high, 3-4 mm wide, glabrous, the bracts imbricate in 2-4 series. **Receptacle** ca 2 mm across, alveolate, glabrous **Floret**s ca 15 per head; corollas "white," glabrous. **Achenes** ca 1.5 mm long, glabrous; pappus of 40-45 white bristles, 3-4 mm long.

TYPE: **MEXICO. OAXACA: Distrito Juchitan, Mpio. San Miguel Chimalapa,** "Benito Juarez, rumbo a cerro Salomon," ca 1710 m, 7 Dec 2008, *Jose Luis Lucas 146* (Holotype TEX).

The name is derived from an acronym of Sociedad para el Estudio de los Recursos Bioticos de Oaxaca.

Bartlettina serboana is closely allopatric with its seemingly closest relative, *B . pinabetense*, as noted in the above diagnosis.

BARTLETTINA SORDIDA (Less.) King & H. Rob., Phytologia 22: 161. 1971. **Map 7**
Bartlettina brevipetiolata (Sch.-Bip. ex Klatt) King & H. Rob.
Bartlettina matudinae King & H. Rob.
Eupatorium megalophyllum (Lem.) Klatt
Eupatorium miradorense Hieron.
Eupatorium raffillii Hemsl.
Eupatorium sordidum Less.
Eupatorium thesesiifolium DC.

Mex, Mor, Pue, Ver, Gue, Oax and Cps in montane cloud forests and lower seasonal evergreen forests, 600-200 m; Dec-Apr.

Perennial, robust herbs or shrubs 1-3 m high; stems pithy, terete, densely soft-velutinous with purplish or brownish hairs; leaves opposite, 10-30 cm long, 4-20 cm wide; petioles 2-10 cm long; blades exceedingly variable, broadly ovate to cordate or elliptic-ovate, (3)5-7-nervate from below, velutinous-pubescent beneath, inconspicuously pellucid-punctate, the margins irregularly dentate to entire; heads purple, numerous, in terminal, rounded, corymbose panicles, ca as wide as long; involucre campanulate, 3-5 seriate, graduate, densely pubescent; receptacle convex to plane, glabrous to pubescent; florets (50)75-200, the lobes glabrous (rarely a few hairs); achenes 1.5-2.0 mm long, glabrous or a few hispid hairs near apex, the pappus of 30-40 bristles, 4-6 mm long, the apices not enlarged; chromosome number, *n* =16 pairs.

This is an extremely variable species, taken into cultivation early-on and introduced into tropical regions the world over where it occasionally escapes. In Mexico the species is native from Ver southwards to Cps, but the variation in this region might now be confounded with escaped cultivars. *Bartlettina matudinae,* the type from Acultzingo, Ver, is a form of the species with broad blades abruptly tapering upon the petiole. Indeed, as noted in the above description, leaf shape is exceptionally variable, even within a single population.

BARTLETTINA TAMAULIPANA (B.L. Turner) King & H. Rob., Phytologia 65: 63. 1988. **Map 8**
Eupatorium tamaulipanum B.L. Turner

Tam, San and Hid, montane cloud forests, 1500-1700 m; Dec-Feb.

Suffruticose perennial herbs or subshrubs to 3 m high; stems softly appressed-pubescent; leaves opposite, 10-20 cm long, 2.5-5.5 cm wide; petioles 1-2 cm long; blades elliptic, pinnately nerved, densely soft-pubescent and atomiferous-glandular beneath; the margins weakly dentate to entire or nearly so; heads purple, 6-numerous in terminal, pyramidal, corymbose panicles; involucres hemispheric, 4-5 seriate, imbricate, the bracts thin, linear-lanceolate, acuminate; receptacle convex, sparsely pubescent; florets numerous, the corollas glabrous;

achenes ca 2 mm long, glabrous, the pappus of 40-50 bristles, 4-5 mm long.

An anomalous species, closely related to *B. xalapana*, but readily distinguished by its somewhat larger heads with pubescent receptacles. These two taxa are not readily related to yet other taxa of the Eupatorieae. On total characters they appear as closely related to the *Chromolaena* complex as perhaps any, but involucral and receptacular characters suggest a relationship with the *Hebeclinium* and *Bartlettina* groups.

BARTLETTINA TUERCKHEIMII (Klatt) King & H. Rob., Phytologia 22: 162. 1971. **Map 8**
Eupatorium tuerckheimii Klatt
Neobartlettina tuerckheimii (Klatt) King & H. Rob.

e Oax, Cps, adjacent Guatemala and Honduras; montane cloud forests with *Quercus* and *Liquidambar*, 1500-3000 m; Dec-Apr.

Suffruticose robust, glabrous herbs or shrubs 1-5 m high; leaves firm, pinnately veined, 10-20 cm long, 2-4(5) cm wide; petioles 1-2(3) cm long; blades lance-ovate to narrowly elliptic, about equally narrowed at both ends, without punctations, the margins undulate with remote apiculations; heads lavender, thimble-shaped, numerous in a terminal corymbose panicle 2-3 times as wide as high; involucres 3-4 seriate, evenly gradate, 5-6 mm high; receptacles plane to convex, 2.5-3.5 mm across, prominently pubescent with hairs 0.5-1.5 mm long; florets 75-150 per head, the corollas tubular, the lobes densely pubescent; achenes 1.5-2.0 mm long, glabrous, the pappus of 35-40 bristles, 4-5 mm long, the apices not enlarged; chromosome number, *n* = 16 pairs.

This is a common species in Cps, well represented in herbaria and easily recognized by its numerous-flowered heads, glabrous foliage, narrow, pinnately veined, leaves and prominently pubescent receptacles.

BARTLETTINA XALAPANA (B.L. Turner) B.L. Turner, Phytologia 82: 306. 1998. **Map 8**
Eupatorium xalapanum B.L. Turner

Known only from Ver in barrancas near Xalapa; Mar-Apr.

Shrub to 2 m high with terminal, numerous-headed, stiffly-pyramidal, capitulescences; superficially very similar to *B. tamaulipana* but the heads smaller, having pubescent receptacles, and the pappus bristles more numerous, more ciliate, and shorter (3-4 mm long vs 4-5 mm); chromosome number, *n* =10 pairs.

The species is locally common below Puente Sedeno in the small barranca along the western edge of Xalapa. King & Robinson (1987) placed this taxon and *B. tamaulipana* in synonymy with their concept of *B. brevipetiolata*, which I consider to be a synonym of *B. sordida*. *Bartlettina tamaulipana* is difficult to position among the segregates of *Eupatorium* (s.l.). King & Robinson (1988) subsequently recognized *B. tamaulipana* as valid, but retained *B. xalpana* as synonymous with the latter. I originally positioned both species within the *Chromolaena* complex of *Eupatorium* (Turner 1997), but subsequently transferred these to *Bartlettina*.

BARTLETTINA YAHARANA B.L. Turner, **sp. nov. Fig. 3, Map 8**

Bartlettinae hastiferae Standley & Steyerm. similis sed foliis majoribus nervis principalibus 5-9 (vs. 3) et capitulis majoribus bracteis involucralibus imbricatioribus flosculis numerosioribus (+ 50 vs. 10-20) differt.

Resembling *Bartlettina hastifera* Standley & Steyerm. but the leaves, larger with 5-9 principal nerves (vs 3), and the heads larger, more imbricate, with more numerous florets (over 50 vs 10-20.

Robust perennial herbs, subshrubs or possibly clambering vines. **Stems** terete, 4-5 mm across, glabrous or nearly so. **Leaves** opposite, 15-20 cm long, 20-30 cm wide, 5-9 palmately nervate from or near the base; petioles perfoliate, 6-8 cm long, callous-winged throughout; blades broadly cordate with 5-7 prominent lobes, between these an irregular serration, the surfaces glabrous. **Capitulescence** a terminal, numerous-headed corymbose panicle ca 25 cm long, 20 cm across, the ultimate peduncles puberulent, 2-5 mm long. **Involucres** campanulate, ca 6 mm high, 6 mm across, the bracts imbricate in 4-6 series.

Receptacle convex, ca 1.5 mm across, glabrous. Florets ca 80 per head. **Corollas** lavender, slender or filiform, glabrous, 4-5 mm long, the throat indistinct; lobes 5, ca 0.2 mm long, atomiferous glandular. **Stamens** with broadly ovate apical appendages, broader than long. **Achenes** glabrous, 4-5 ribbed, ca 1.5 mm long; pappus of ca 40 readily deciduous slender white bristles ca 4 mm long.

TYPE: **MEXICO. OAXACA: Mpio. San Juan Lachao,** "25 km N of Mixtepec, on the way from Puerto Escondido to Oaxaca. Half-shaded rocky slope along a stream, upstream of Rio Salado, 1355 m." latitude given as 16 11.6 N, 13 Nov 1997, *T. Yahara, M. Mishima, T. Kajita & K. Ooi 1129* (Holotype: TEX).

Among Mexican species this is a very distinct taxon, what with its very large, palmately nervate leaves, and many-flowered heads, resembling nothing previously collected in that country. Vegetatively, it most resembles the Guatemalan species, *B. hastifera*, with which it is compared in the above diagnosis.

The eponym derives from Prof. T. Yahara of Kyushu Univ., Fukuoka, Japan, principal collector of the type, and exceptional field collector and scholar of the genus *Stevia* (cf. numerous papers in collaboration with his colleagues, these mostly cited in Watanabe et al. (2001),

ACKNOWLEDGEMENTS

LITERATURE CITED

See citations listed under above **REFERENCES**.
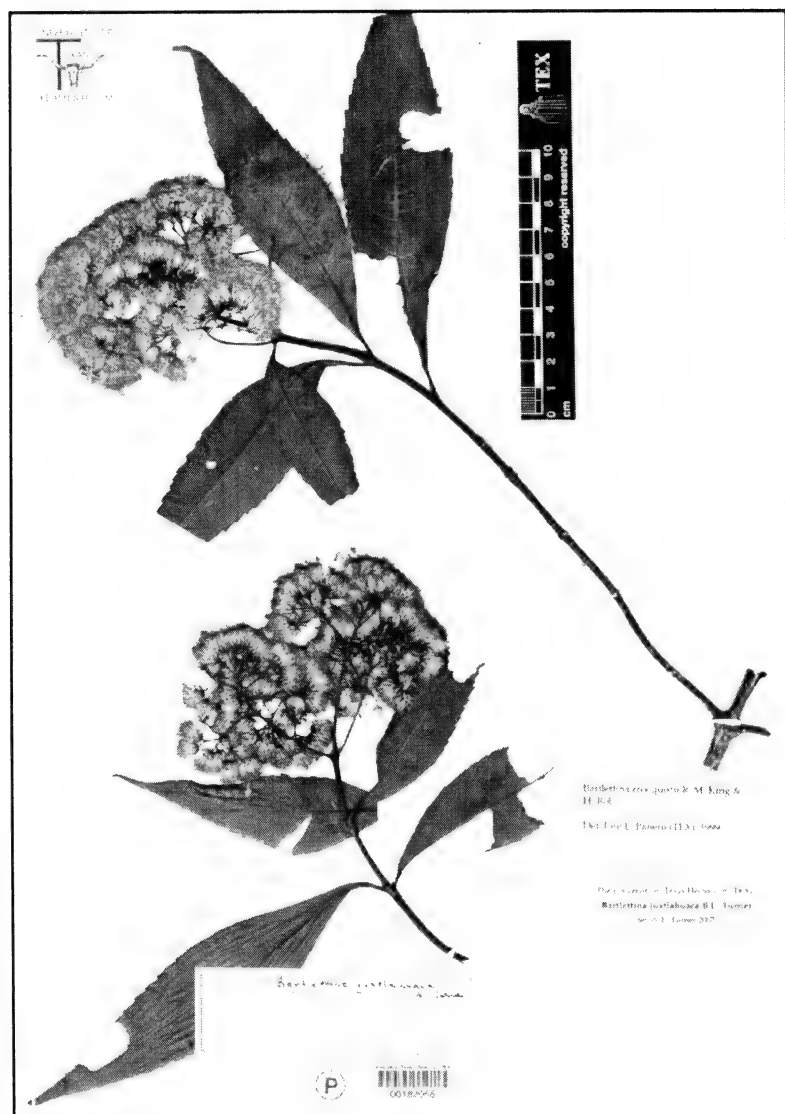
Fig. 1. *Bartlettina juxtlahuaca*, holotype.
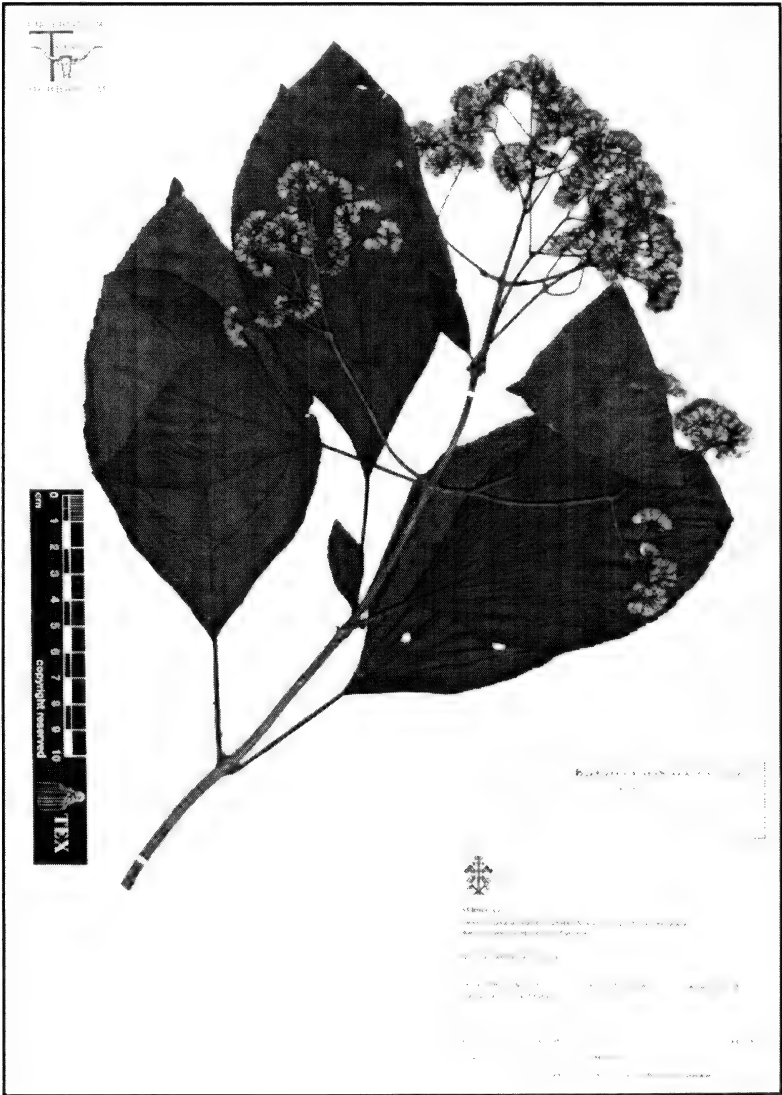
Fig. 2. *Bartlettina serboana.* holotype.
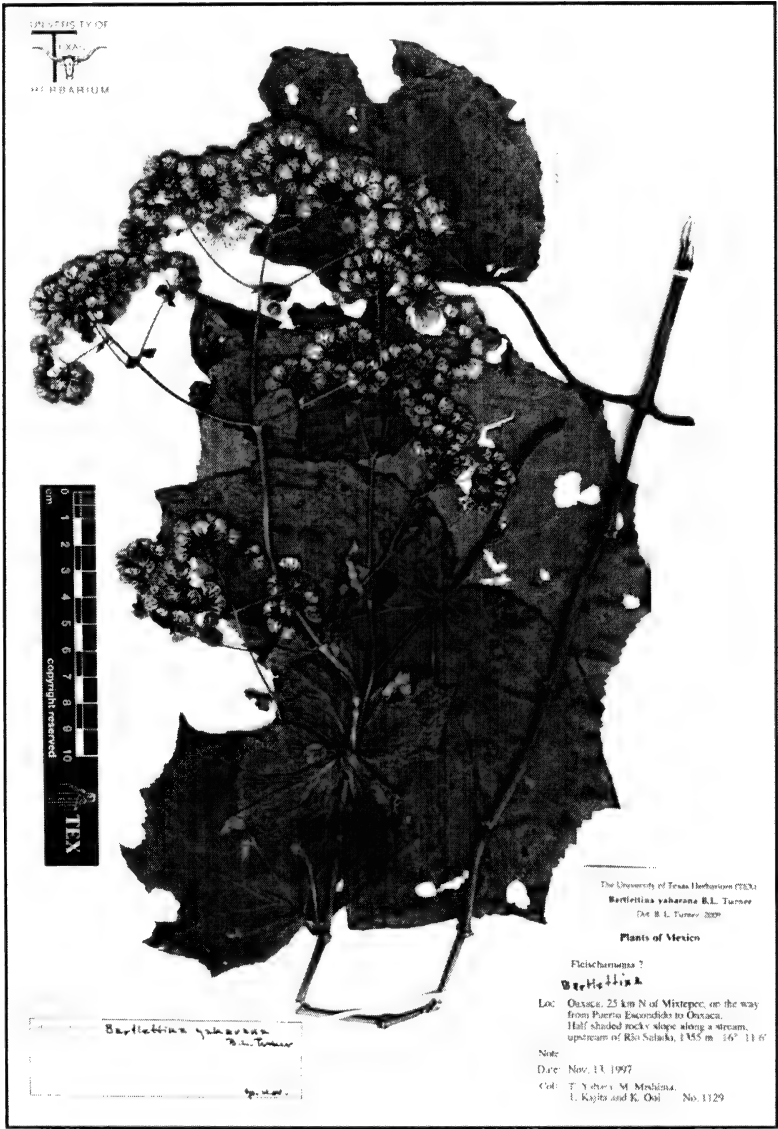
Fig. 3. *Bartlettina yaharana*, holotype.

BARTLETTINA
- ○ breedlovei
- ● **calderonii**

Map 1

BARTLETTIA
- ● constipatiflora
- ● cronquistii
- ○ ehrenbergii

Map 2

BARTLETTINA
o hintonii
• juxtlahuaca

Map 3

BARTLETTIA
● karwinskiana
• lanicaulis
O macdougalii

Map 4

BARTLETTINA
- oresbia
- **ornata**
- pansamalensis

MAP 5

BARTLETTINA
- pinabetensis
- platyphylla

MAP 6

BARTLETTINA
○ serboana
● sordida

MAP 7

BARTLETTINA
○ tamaulipana
● tuerckheimii
• xalapana
★ yaharana

MAP 8

THE ENIGMATIC GENUS *PYTHIELLA* (OOMYCOTA)

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ABSTRACT

Pythiella (Oomycetes, Stramenopiles) is a small genus of hyperparasitic organisms (parasitic in other parasitic Oomycetes). These obscure organisms may escape notice, and are probably more common than reports suggest. *Pythiella* and its original species, *P. vernalis*, are technically nomenclaturally invalid; no Latin diagnoses were provided with original descriptions (Couch, 1935). Biological validity of organisms identified as *Pythiella* should, though, be further established before effecting nomenclatural validity. Four putative species of *Pythiella* are known, although the most recently described of these has not been named. These taxa exhibit a similar, achlyoid ("ball-like") mode of spore discharge; sporangia are illustrated herein. The history of *Pythiella* is reviewed in hope of fostering interest in these puzzling organisms. A key to species is presented—a preliminary guide, given the relatively meager systematic information available to this point. *Phytologia 92(3) 304-311 (December 1, 2010).*

Pythiella species are poorly known Oomycetes—difficult to find, not obtainable from culture collections, tedious to manipulate (not yet cultured without hosts); they are holocarpic (the thallus completely converted to asexual or sexual reproductive structures). Sequence data and ultrastructural information are lacking. However, enough details of morphology are known—knowledge admittedly mixed with an array of questions—that it is possible to offer a taxonomic synopsis. Zoospore behavior—systematically significant among Oomycetes (cf. Powell and Blackwell, 1998; Blackwell and Powell, 2000)—appears to unify the genus. Perhaps this presentation will promote additional discoveries of the life cycles of organisms assigned to *Pythiella*.

TAXONOMIC HISTORY OF *PYTHIELLA*

In 1935, Couch described *Pythiella* (lacking Latin diagnosis), a genus seemingly combining features of several oomycete genera: *Olpidiopsis*, *Aphanomycopsis*, *Pythium*, *Ectrogella*, *Achlya* and *Saprolegnia*. *Pythiella* was of further interest in that it parasitized hyphae of *Pythium* (*P. gracile* and *P. dictyosporum*) which in turn parasitized filaments of *Spirogyra*. Microscopically obvious swellings or "galls" in threads of *Pythium* contained *Pythiella*—either a spherical sporangium (Fig. 1) with sometimes elongate, occasionally branched exit tubes, or a single-spored oogonium with an antheridium attached by a small fertilization tube (Fig. 5). The mode of spore discharge, in which internally cleaved spores aggregate, externally, in a ball-like cluster of primary cysts—at or near the opening of a sporangial discharge tube—reminded Couch of the saprolegniaceous genus, *Achlya*. Secondary, swarming, biflagellate zoospores developed from the primary cysts. A single species exhibiting these sporangial and oogonial features, *Pythiella vernalis* (also lacking Latin diagnosis), was recognized by Couch. As the only original species of *Pythiella* (Couch, 1935), *P. vernalis* is the type of the genus; Couch reported depositing preserved slide material at the University of North Carolina Herbarium. Among oomycete genera, Couch believed *Pythiella* to have its closest relationships to *Ectrogella* and *Aphanomycopsis*, despite certain differences in appearance of the protoplasm (cf. Couch, p. 167).

Sparrow (1943) initially considered *Pythiella* somewhat similar to *Olpidiopsis*, and placed it in the Lagenidiales (Oomycetes). Sparrow and Ellison (1949) described a parasite of *Olpidiopsis schenkiana* (itself a parasite of *Spirogyra*), found in Michigan, which they named *Ectrogella besseyi*. Sparrow and Ellison indicated that Scherffel (1925) and Domján (1936) had (wittingly or not) figured an organism recognizable as *Ectrogella besseyi* in drawings of *Olpidiopsis* (see, for example, Domján, Fig. 173). Although possessing a more asymmetric thallus (Fig. 2)—sometimes lobed or tubular (in some cases more than one thallus being present in an old *Olpidiopsis* cell)—Sparrow and Ellison (1949) mentioned a resemblance of certain other features (e.g., an achlyoid mode of spore discharge) of *E. besseyi* to Couch's *Pythiella vernalis*. Sparrow (1960) later became convinced of a closer relationship of *E. besseyi* to *Pythiella* than to *Ectrogella*, and effected

transfer of this species, viz. *Pythiella besseyi* (Sparrow & Ellison) Sparrow. In considering Orders of Oomycetes, Sparrow (1960) concluded that *Pythiella*—given its zoospore behavior, and a general relationship to *Ectrogella*—had its connections with the Saprolegniales rather than the Lagenidiales.   Unfortunately, details of sexual reproduction of *Pythiella besseyi* remain unobserved, leaving the relationship of this organism still somewhat in question.

Whiffen (1946) described an *Olpidiopsis*-like species of *Lagenidium*, *L. pythii*, found in *Pythium* sp., growing on grass-leaf bait in a water culture of a soil sample from Cuba.   Several spherical or flask-like sporangia (Fig. 3)—each with a single, short, relatively stout exit tube—were observed in club-like swellings of the *Pythium* host; other swellings contained oogonia or resting spores; in some instances, sporangia and resting spores occurred in the same swelling.   Whiffen noted that spore discharge in *L. pythii* was anomalous for *Lagenidium* [being more similar to *Achlya*]; nonetheless, Whiffen placed this organism in *Lagenidium*.   Sparrow (1960) and Karling (1981) continued to recognize this *Pythium* parasite as a species of *Lagenidium*.   However, Dick (2001, p. 245, 337) concluded that *Pythiella* should include this taxon.   The combination *Pythiella pythii* (Whiffen) Dick should date from Dick (2001, p. 337)—not Dick (1998), as Dick (2001, p. 245) appeared to imply. *Index Fungorum* and *Index Nominum Genericorum* correctly cite Dick (2001) for the combination *Pythiella pythii*.

Dick (2001, p. 244-245) considered *Pythiella* a genus "Insertae Sedis," and stated that *Pythiella* (i.e., the type species, *P. vernalis*)— though definitely an oomycete, with laterally biflagellate zoospores— "does not have unequivocal affinity" to any particular known oomycete order.   Dick doubted the biological authenticity of *Pythiella besseyi*, considering its alleged occurrence an example of "autoparasitism" by *Olpidiopsis schenkiana*.   Dick (2001, p. 337) excluded *Pythiella besseyi* from *Pythiella*.   However, illustrations by Sparrow and Ellison (1949) and Domján (1936)—and my observations of what was apparently this organism in *Olpidiopsis* occurring in *Spirogyra* in central Alabama— suggest not only that *Pythiella besseyi* exists, but that its morphology is distinctive from *Olpidiopsis*. As a further point of confusion, Dick (2001, p. 245, 337) stated that he placed this organism (*Pythiella*

besseyi) in *Pleocystidium*, and is credited with the combination (*Pleocystidium besseyi*) in *Index Nominum Genericorum*; this combination is also listed, minus authorship, in *Index Fungorum*. Dick (2001), though, did not make this combination under *Pleocystidium* (p. 345-346), or in his discussion on p. 245. Dick (p. 345) was apparently merely suggesting that *Olpidiopsis schenkiana* be included in the synonymy of *Pleocystidium* (under *P. parasiticum*). Dick (2001) did list the combination "*Pleocystidium besseyi* (F. K. Sparrow & B. Ellison) M. W. Dick" on p. 337; however, this does not seem to be where the combination was effected; in the index of his book Dick refers (this combination) to p. 236, but it does not appear on this page.

It is difficult to know how common *Pythiella* is, as it surely often goes unnoticed as an inconspicuous parasite of other oomycete parasites (even these are not always readily detected). Czeczuga (1991) reported *Pythiella vernalis* from a lake in Poland. Pires-Zottarelli et al. (2009) documented *Pythiella vernalis* in Brazil, in a different *Pythium* host (*P. aphanidermatum*) than previously reported. Kühn and Schnepf (2002) reported "*Pythiella* sp." parasitizing a *Lagenidium* parasitic in *Glaucocystis nostochinearum* from a freshwater pond in northern Germany. This unnamed *Pythiella*—relatively smaller than *P. vernalis* (the species mainly compared by Kühn and Schnepf)—is not identical in sporangial morphology to other taxa of *Pythiella*, being often ovoid and possessing a more slender, irregular, discharge tube (Fig. 4); this tube is not branched (as it sometimes is in *P. vernalis*), and multiple tubes (per sporangium) do not occur (as they often do, for example, in *P. besseyi*). In spite of differences, achlyoid spore discharge was observed. Primary zoospores of this *Pythiella*, described by Kühn and Schnepf, very briefly exhibit flagella before encysting; typical swarming, laterally biflagellate secondary zoospores are then observed. In other *Pythiella* species, only one flagellated stage is seen, i.e., the equivalent of the secondary zoospore stage; a primary cyst stage was, however, illustrated for *P. vernalis* by Couch (1935).

GENERIC DESCRIPTION, KEY TO SPECIES

Below is quoted the original generic description of *Pythiella*, given by Couch (1935) in English but not Latin. Since the rule (Article 36.1, ICBN, 2006) requiring a Latin description or diagnosis took effect

January 1, 1935, *Pythiella* is (by "the letter of the law") an invalid name, as is the species name, *P. vernalis*. Should *Pythiella* prove to be a biologically viable genus, it will require nomenclatural validation, even if current attempts to repeal the "Latin Rule" succeed (cf. Figueiredo et al., 2010); i.e., such a change would not be retroactive. Following Couch's description, I present a preliminary key to the four supposed species of *Pythiella*, including *Pythiella* sp. (Kühn and Schnepf, 2002).

Description of *Pythiella* by Couch, 1935

"Plant body parasitic within the threads of *Pythium*; without rhizoids, the entire thallus upon maturity being transformed into reproductive organs. Spore development as in the higher water fungi (*Achlya* and *Saprolegnia*, e.g.). Spores after emergence encysting at the tip of the sporangium as in *Achlya*, swarming later in the laterally biciliate condition. Antheridia present on all oögonia. Egg not completely filling the oögonium, and with a distinct periplasm."

Preliminary Key to Species of *Pythiella*

1. Occurring in *Pythium*. Sporangium generally spherical or flask-shaped; discharge tube branched or unbranched.

   2. *Pythium* host parasitic in *Spirogyra*. Sporangium typically one per host "gall" or swelling, occasionally more; discharge tubes may be more than one per sporangium, often longer than sporangium, somewhat irregular, sometimes branched...
   ....*Pythiella vernalis* Couch, 1935

   2. *Pythium* host occurring on vegetable matter, not in *Spirogyra*. Typically more than one sporangium found in host "gall;" discharge tube one per sporangium, shorter than sporangium, stout and regular, unbranched......*Pythiella pythii* (Whiffen) Dick, 2001

1. Occurring in hosts other than *Pythium*. Sporangium shape variable, often ovoid or asymmetric; discharge tube unbranched.

   3. In *Olpidiopsis*, which in turn occurs in *Spirogyra*. Sporangium 13-

42μm, often somewhat asymmetric, sometimes tubular or lobed; discharge tubes often more than one per sporangium, usually less than sporangial length, more or less regular; one motile zoospore stage...........*Pythiella besseyi* (Sparrow & Ellison) Sparrow, 1960

3. In *Lagenidium*, in turn found in *Glaucocystis*. Sporangium 12-17 μm, ovoid to spheroid; discharge tube slender, irregular, often becoming greater than sporangial length, only one present per sporangium; primary zoospores (briefly) and secondary zoospores developing flagella..*Pythiella* sp.
(described but unnamed, Kühn and Schnepf, 2002)

ACKNOWLEDGMENTS

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Fig. 1 (based on Couch, 1935): Sporangium (arrow) of *Pythiella vernalis*, in swollen area of *Pythium* hypha (in *Spirogyra*, plastid remnants evident). **Fig. 2** (after Sparrow and Ellison, 1949): Sporangium of *Pythiella besseyi* (arrow) in old *Olpidiopsis* cell (in *Spirogyra*). **Fig. 3** (after Whiffen, 1946): Sporangia (arrows) of *Pythiella pythii*, in *Pythium*. **Fig. 4** (based on Kühn and Schnepf, 2002): Sporangium (arrow) of *Pythiella* sp. in *Lagenidium* (in *Glaucocystis*). **Fig. 5** (based on Couch, 1935): *Pythium* hyphae (in *Spirogyra*); "gall" with oogonium (arrow) of *Pythiella vernalis*; antheridium attached (opposite arrow). Another gall (to right) shows cleaved primary spores.
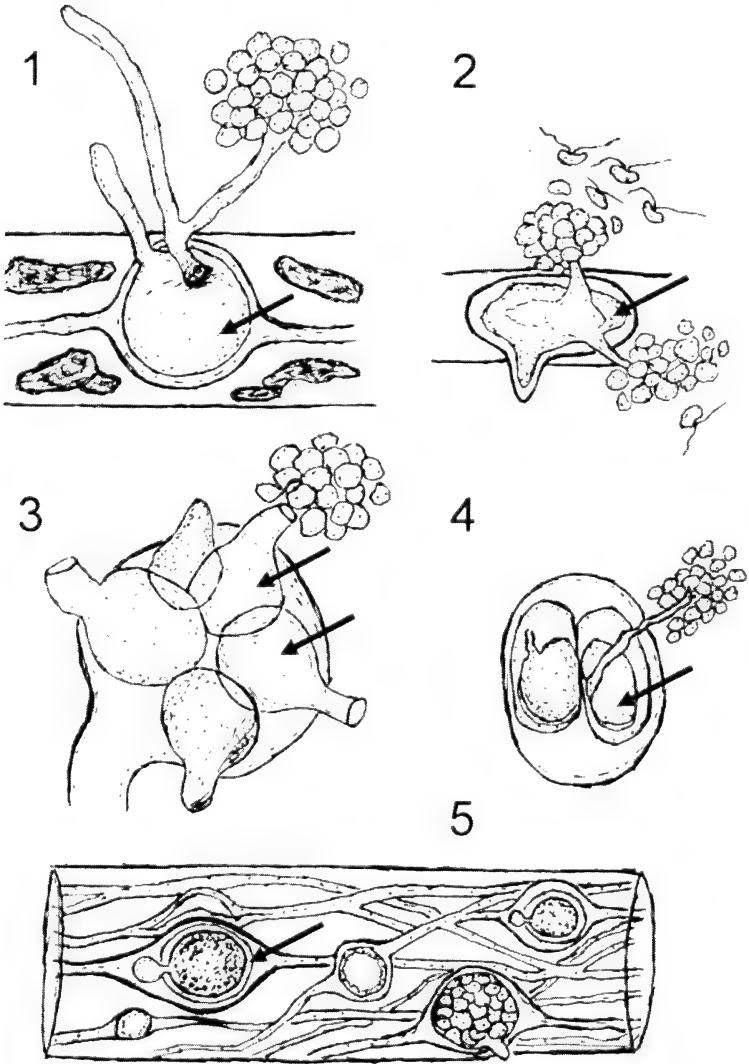
Figures 1-5, see caption on previous page.

CHROMOSOME COUNTS OF *BIDENS*, *COSMOS* AND *THELESPERMA* SPECIES (ASTERACEAE, COREOPSIDINAE)

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ABSTRACT

Chromosome counts are presented for *Bidens*, *Cosmos*, *Thelesperma* species. *Phytologia 92(3): 312-333 (December 1, 2010).*

KEY WORDS: *Bidens*, *Cosmos*, *Thelesperma*, chromosome counts, Coreopsidinae.

The chromosome counts presented here were made from pollen mother cell squashes of materials collected throughout Mexico, the southwestern U.S. (primarily Arizona and New Mexico), and Central America (primarily Guatemala). Nos. 6006-6240 were collected by T. E. Melchert and P. D. Sørensen in 1965; Nos. 6251-6504 by T. E. Melchert, P. D. Sørensen and D. J. Crawford in 1966; Nos. 67-1 through 67-182 by T. E. Melchert, D. J. Crawford and J. E. Averett in 1967; Nos. 68-1 through 68-46 by N. J. Carman in 1968; Nos. 68-48 through 68-138 by N. J. Carman and D. E. Giannasi in 1968; Nos. 71-1 through 71-21 by C. R. Hart in 1971; Nos. 71-22 through 71-308½ by T. E. Melchert, C. R. Hart and R. E. Ballard in 1971; and those with a (R-) prefix by R. R. Roseman in 1976.

The methods used to obtain and document these counts are those of Melchert (1968). In the following tables counts obtained from individual plants are distinguished by letters (A, B, C, D) appended to the population collection number. Counts obtained from progeny grown from seed in The University of Iowa greenhouses are marked by the symbol (GH). The chromosome numbers presented here for the populations comprising the "*Bidens pilosa* species complex" (*B.*

odorata, *B. alba*, *B. pilosa* and *B. clavata*) collected in 1971 were determined by Ballard (1975, 1986, 1988).

Voucher specimens are deposited in The University of Texas Herbarium (TEX). These studies were supported by N.S.F. grants GB-3851 and GB-6684.

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TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens acrifolia Sherff		
MEXICO:		
Jalisco:	$n = 10$	6376, 6385, 68-113
Bidens aequisquama (Fernald) Sherff		
MEXICO:		
Jalisco:	$n = 36$	6407
	$n = $ ca 36	6389, 68-59
Michoacán:	$n = 36$	68-126, 68-128, 247
Bidens aequisquama var. *guanajuatensis* Melchert		
MEXICO:		
Guanajuato:	$n = 36$	71-94 (Type collection)
Bidens alba (L.) DC. var. *alba*		
MEXICO:		
Veracruz:	$n = 24$	71-127, 71-130, Cruden 1893
Bidens alba var. *radiata* (Sch.-Bip.) Ballard		
MEXICO:		
Campeche:	$n = 24$	71-143B, Cruden 1912
Oaxaca:	$n = 24$	68-35, 71-137
Tamaulipas	$n = 24$	King 4058
Veracruz	$n = 24$	71-124, 71-125B, 71-128, 71-129, 71-130, 71-131, 71-133, 71-134, 71-136, Cruden 1913

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens angustissima H. B. K.		
MEXICO:		
Durango:	$n = 10$	6290, Cruden 1855
Guanajuato:	$n = 10$	71-100
Hidalgo:	$n = 10$	*Beaman 2763 (TEX)
San Luis Potosi:	$n = 10$	71-72
Bidens anthemoides (DC.) Sherff		
MEXICO:		
Mexico D. F.:	$n = 11$	67-126
Mexico:	$n = 11$	71-115 (GH), 71-234
*Bidens aurea (*Ait.) Sherff		
MEXICO:		
Mexico:	$n = 17$ or 18	67-164
Chiapas:	$n = 23$	6460A, 6460B, Cruden 1543C, Cruden 1543D, Cruden, 1537, Cruden 1548, Cruden 1549
Durango	$n = 23$	6291, 68-85
Guanajuato:	$n = 23$	67-175
Hidalgo:	$n = 23$	67-41, 67-38
Jalisco:	$n = 23$	71-81A, 71-267, 71-277A, 71-277B, 71-277C

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: **State**	**Taxon**	**Collections**
*Bidens aurea (*Ait.) Sherff (cont'd)		
MEXICO (cont'd):		
Mexico:	$n = 23$	6115, 67-138, 67-147, 67-166, 67-150, 6230, 71-109
Michoacán:	$n = 23$	67-167A, 67-167B, 67-167C, 67-168, 6402, 67-174B, 67-175, 6413
Oaxaca:	$n = 23$	6191, 67-107, 68-32, 68-32B, 68-37
Puebla:	$n = 23$	6150
San Luis Potosi:	$n = 23$	67-16
Tlaxcala:	$n = 23$	67-46
Veracruz:	$n = 23$	67-114
Michoacán:	$n = 34$ or 35	6098, 71-244
(Mirador:	$n = $ ca 35 (34-36)	67-170
Mil Cumbres:)	$n = $ ca 35 (or 36)	71-244
Bidens balsana Melchert		
MEXICO:		
Guerrero:	$n = 11$	67-154, 71-206
Bidens bicolor Greenm.		
MEXICO:		
Chiapas:	$n = 12$	6455, 71-150, 71-154

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens bicolor Greenm. (cont'd)		
MEXICO (cont'd):		
Oaxaca:	$n = 12$	71-172
GUATEMALA:	$n = 12$	6477, R-580
Bidens bigelovii A. Gray		
MEXICO:		
Chiapas:	$n = 24$	6454
Durango:	$n = 24$	6287
Guanajuato:	$n = 24$	71-96, 71-97, 71-98
Jalisco:	$n = 24$	71-274 (GH)
Querétaro:	$n = 24$	67-27
San Luis Potosi:	$n = 24$	67-21, 71-55, 71-56A, 71-56B
UNITED STATES:		
Arizona:	$n = 24$	71-12, 71-15
New Mexico:	$n = 24$	71-4, 71-6, 71-8
Bidens carpodonta Sherff		
MEXICO:		
Nuevo Leon:	$n = 12$	6 counts, Hart 1979
Bidens chiapensis Brandegee		
MEXICO:		
Chiapas:	$n = 12$	6466, 71-148

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens chiapensis Brandegee (cont'd)		
GUATEMALA:	$n = 12$	6436
Bidens chrysanthemifolia (H. B. K.) Sherff		
MEXICO:		
Chiapas:	$n = 24$	71-146A, 71-146B
GUATEMALA:	$n = 24$	R-581, 6440A, 6442, 6445
Bidens clavata Ballard		
MEXICO:		
San Luis Potosi:	$n = 12$	71-52. 71-57, 71-60, 71-62, 71-63A, 71-64A, 71-64B
San Luis Potosi:	$n = 12 +$ $(n = 14)$	71-63B (71-63A is $n = 12$)
Bidens cronquistii (Sherff) Melchert		
MEXICO:		
Guerrero:	$n = 12$	71-210
Bidens ferulaefolia (Jacq.) DC.		
MEXICO:		
8 states:	$n = 12$	22 counts, Hart 1979
Bidens laevis (L.) B. S. P. Presl.		
MEXICO:		
Jalisco:	$n = 12$	6345
Bidens lemmonii A. Gray		
MEXICO:		
Chihuahua:	$n = 12$ (GH)	71-307 ½

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens leptocephala Sherff		
MEXICO:		
Chihuahua:	$n = 11$	6254A, 6254B
UNITED STATES:		
Arizona:	$n = 11$	71-9, 71-14, 71-16
Bidens longistyla Hart		
MEXICO:		
Coahuila:	$n = 12$	5 counts, Hart 1979
Bidens mollifolia Sherff		
MEXICO:		
Durango:	$n = $ ca 24	6310
	$n = 36$	6307, 68-23, 71-291
Jalisco:	$n = 24$	71-266
Mexico:	$n = 36$	71-232
Michoacán:	$n = 24$	68-27, 68-29, 71-245
Oaxaca:	$n = 24$	6188A, 6188B, 67-78, 67-101, 67-104, 71-184
	$n = 36$	71-173
Bidens oaxacana Melchert		
MEXICO:		
Oaxaca:	$n = 12$	67-87
Bidens ocellatus (Greenm.) Melchert		
MEXICO:		
Mexico D. F.:	$n = 12$	6485, 6485A, 67-131, 71-222

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: **State**	**Taxon**	**Collections**
Bidens odorata Cav. var. *chilpancingensis*		
MEXICO:		
Guerrero:	$n = 12$	71-217
	$n = 12, 13$	71-207
Bidens odorata Cav. var. *odorata*		
MEXICO:		
Aguascalientes:	$n = 12$	68-97, 71-85
Chiapas:	$n = 12$	6461A, 6461C, 71-152, 71-158
Chihuahua	$n = 12$	6253A, 6253B, 6257, 6262A, 6262B, 68-72, 68-73, 68-74, 68-75
Coahuila:	$n = 12$	71-23, 71-25, 71-32
Durango:	$n = 12$	67-76, 68-94, 68-95, 71-289, 71-294, 71-297
Guanajuato:	$n = 12$	67-22, 67-24
Guerrero:	$n = 12$	67-153
Hidalgo:	$n = 12$	67-33, 67-36
Mexico:	$n = 12$	6114, 6127, 6491, 67-118, 67-134, 67-159, 67-162A, 67-162B, 68-52, 68-53, 71-229, 71-231, 71-241
Michoacán:	$n = 12$	6420, 71-242

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: **State**	**Taxon**	**Collections**
Bidens odorata Cav. var. *odorata* (cont'd)		
MEXICO (cont'd):		
Morelos:	$n = 12$	67-30
Nuevo Leon:	$n = 12$	71-35, 71-40, 71-47
Puebla:	$n = 12$	71-118, 71-120, 71-204
Querétaro:	$n = 12$	67-24, 67-30, 68-104, 71-103, 71-105, 71-107
San Luis Potosi:	$n = 12$	68-103, 71-48, 71-49, 71-70
Tlaxcala	$n = 12$	67-47
Zacatecas:	$n = 12$	71-26, 71-77, 71-281, 71-283
GUATEMALA:		
Huehuetenango:	$n = 12$	6450
Quezaltenango:	$n = 12$	6448
UNITED STATES:		
New Mexico:	$n = 12$	71-3
Bidens odorata var. *rosea* Melchert		
= *B. o.* var. *calcicola* (Greenm.) Ballard		
MEXICO:		
Chiapas:	$n = 12$	71-139, 71-141
Colima:	$n = 12$	71-258

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: **State**	**Taxon**	**Collections**
Bidens odorata var. *rosea* Melchert (cont'd)		
= *B. o.* var. *calcicola* (Greenm.) Ballard (cont'd)		
MEXICO (cont'd):		
Durango:	$n = 12$	6278, 71-295, 71-296
Guanajuato:	$n = 12$	67-176A
Jalisco:	$n = 12$	6361, 6366A, 71-88, 71-264, 71-268, 71-280
	$n = 12 +$ $n = $ ca 13	6061, 6392
	$n = 12, 13 +$ $(13 + I)$	71-278
Mexico:	$n = 12$	67-151, 71-226
Michoacán:	$n = 12$	6403, 68-26, 68-125, 71-246, 71-253, 71-254, 71-255A, 71-255B
Morelos	$n = 12$	71-221
Nayarit	$n = 12$	6334, 6334A
Oaxaca:	$n = 12$	71-138, 71-164, 71-170
San Luis Potosi:	$n = 12$	71-638
	$n = 12 + 14$	71-63B
Bidens odorata var. *oaxacensis* Ballard		
MEXICO:		
Guerrero:	$n = 12$	71-211, 71-218, 71-220

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens odorata var. *oaxacensis* Ballard (cont'd)		
MEXICO (cont'd):		
Mexico:	$n = 12$	71-225, 71-237
Oaxaca:	$n = 12$	67-51, 67-52, 67-57, 67-58, 67-74, 67-105, 67-109, 71-171, 71-181, 71-183, 71-187, 71-192
Puebla:	$n = 12$	67-51, 67-51B, 71-205
Bidens ostruthiodes (DC.) Sch.-Bip.		
MEXICO:		
Chiapas:	$n = 23$	6465
Mexico:	$n = 23$	6222, 67-135, 68-31
Michoacán:	$n = 23$	6429, 68-28
Oaxaca:	$n = 23$	67-100
GUATEMALA:	$n = 23$	6435, Cruden 1556, Cruden 1576
Bidens pilosa L.		
MEXICO:		
Chiapas:	$n = 36$	Cruden 1932, R-521 ("var.." *minor*)
Durango:	$n = 36$	6315, 6317, Cruden 1866
Guanajuato:	$n = 36$	*67-176B, *67-176C, 67-177, 68-108

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens pilosa L. (cont'd)		
MEXICO (cont'd):		
Jalisco:	$n = 36$	63-66
Michoacán:	$n = 36$	6082, 6087, 6094, 6416
Oaxaca:	$n = 36$	68-38, 71-175, 71-180A
Querétaro:	$n = 36$	68-106
COSTA RICA:	$n = 36$	R-533 ("var.." *minor*)
Bidens pringlei Greenm.		
MEXICO:		
Michoacán:	$n = 12$	6418, 67-173
Bidens pueblensis (Sherff) Melchert		
MEXICO:		
Mexico:	$n = 12$	71-228
Oaxaca:	$n = 12$	71-185
Bidens purpusorum Biter & Peters		
MEXICO:		
Puebla:	$n = 11$ (GH)	67-115, 71-121, 71-122
	$n = 11$	Powell & Edmundson 641 (originally reported as *B. f. reptans*)
Bidens riparia H. B. K.		
MEXICO:		
Jalisco:	$n = 12$	71-261, 263
PANAMA:	$n = 12$	R-59

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens rostrata Melchert		
MEXICO:		
Jalisco:	*n* = 12	71-273
Michoacán:	*n* = 12	R-508
Bidens schaffneri (A. Gray) Sherff		
MEXICO:		
Aguascalientes:	*n* = 11	71-86
Jalisco:	*n* = 11	6356, 71-80, 71-90
Mexico:	*n* = 11	67-158
San Luis Potosi:	*n* = 11	71-86, 71-75A, 71-75B
Bidens schaffneri var. *wrightii* (Sherff) Melchert		
MEXICO:		
Chihuahua	*n* = 11	6270A, 6270B, 6261
Bidens schaffneri var. *schaffneri* x var. *wrightii*		
Artificial Hybrid	*n* = 11	67-158 ♂ x 6261 ♀ (GH) (meiotic pairing normal)
Bidens serrulata (Poir.) Desf.		
MEXICO:		
Mexico:	*n* = 12	67-45A, 67-45B, 67-45C, 67-148, 71-111, 71-113, 71-114, 71-230, 71-239
Mexico D.F.:	*n* = 12	67-117, 67-120, 67-125A, 67-125B
Morelos:	*n* = 12	71-223

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens serrulata (Poir.) Desf. (cont'd)		
MEXICO (cont'd):		
Puebla:	*n* = 12	71-117
Bidens sharpii (Sherff) Melchert		
MEXICO:		
Oaxaca:	*n* = 11	6481, 6482, 6483, 67-64, 67-66, 67-106, 68-39, 71-178, 71-179, 7!-186, 71-199, 71-201
	n = 11	King 2517 (TEX) (reported as *B. serrulata* var. *sharpii*)
Bidens subspiralis McVaugh		
MEXICO:		
Michoacán:	*n* = 12	R-509
Bidens tenuisecta A. Gray		
UNITED STATES:		
Arizona:	*n* = 12	H-71-1
New Mexico:	*n* = 12	H-71-18
Bidens triplinervia H. B. K.		
MEXICO:		
Chiapas:	*n* = 24	71-151
	n = ca 24	King 2990
Durango:	*n* = 24	6300
Guerrero:	*n* = 12	Cruden 1631 (Cal)
Hidalgo:	*n* = 24	67-37, 67-42, 68-56

TABLE 1. CHROMOSOME COUNTS OF *BIDENS*		
COUNTRY: State	**Taxon**	**Collections**
Bidens triplinervia H. B. K. (cont'd)		
MEXICO (cont'd):		
Mexico:	$n = 24$	6103, 6432A, 67-128, 67-133
Michoacán:	$n = 24$	6424, 67-169, 68-30B
Nuevo Leon:	$n = 24$	67-7A, 67-7B
San Luis Potosi:	$n = 24$	67-18, 71-58
Oaxaca:	$n = 24$	6196, 68-41
Veracruz: .	$n = 24$	Rock 391
GUATEMALA:	$n = 12$	Beaman 3743
	$n = 24$	Beaman 2916 (MSC)
	$n = 36$	Beaman 3283 (MSC), Cruden 1583, Cruden 1575

TABLE 2. CHROMOSOME COUNTS OF *COSMOS*		
COUNTRY: State	**Taxon**	**Collections**
Cosmos section *Cosmos:*		
Cosmos bipinnatus Cav.		
MEXICO:		
Hidalgo:	$n = 12$ II	67-39
Querétaro:	$n = 12$ II	67-31
Tlaxcala:	$n = 12$ II	67-48
Cosmos caudatus H. B. K.		
MEXICO:		
Veracruz:	$n = 24$ II	71-126, 71-132
PANAMA:	$n = 24$ II	R-538
Cosmos pacificus Melchert		
MEXICO:		
Colima:	$n = 12$ II	71-259
Michoacán:	$n = 12$ II	71-124, 71-248, 71-251A, 71-251B
Mexico:	$n = 12$ II	71-236
Cosmos pacificus var. *chiapensis* Melchert		
MEXICO:		
Chiapas:	$n = 12$ II	71-161
Cosmos parviflorus (Jacq.) Pers.		
MEXICO:		
Hidalgo:	$n = 12$ II	67-49
Mexico:	$n = 12$ II	67-161 ½

TABLE 2. CHROMOSOME COUNTS OF *COSMOS*		
COUNTRY: State	**Taxon**	**Collections**
Cosmos parviflorus (Jacq.) Pers. (cont'd)		
MEXICO (cont'd):		
San Luis Potosi:	$n = 12$ II	67-20
Cosmos sulphureus Cav.		
MEXICO:		
Chiapas:	$n = 12$ II	71-162A, 71-162B
Colima:	$n = 12$ II	71-257
Mexico:	$n = $ ca 12 II	71-238
Michoacán:	$n = 12$ II	71-249A, 71-249B, 71-252
Oaxaca:	$n = 12$ II	67-83
Cosmos sulphureus x *C. pacificus* F_1 Hybrids		
MEXICO:		
Chiapas:	$n = $ ca 12 (8II, 1IV, 1III, 1I)	71-160
Colima:	$n = 12$ II (*very irregular*)	71-260
Michoacán:	$n = 12$ II (*very irregular*)	71-250
Cosmos section *Discopoda:*		
Cosmos deficiens (Sherff) Melchert		
MEXICO:		
Jalisco:	$n = 12$	68-114

TABLE 2. CHROMOSOME COUNTS OF *COSMOS*		
COUNTRY: **State**	**Taxon**	**Collections**
Cosmos diversifolius Otto		
MEXICO:		
Hidalgo:	$n = 24$	67-43
Oaxaca:	$n = 12$	67-93, 68-44
San Luis Potosi:	$n =$ ca 24	67-19A, 67-19B, 67-19C, 67-19D
Cosmos jaliscensis Sherff		
MEXICO:		
Jalisco:	$n = 24$	68-16
Cosmos modestus Sherff		
MEXICO:		
Mexico:	$n = 12$	67-139A, 67-139B, 67-140
Cosmos montanus Sherff var. *pinnatus* Sherff		
MEXICO:		
Jalisco:	$n = 24$ (GH)	68-119
Cosmos palmeri B. L. Robinson		
MEXICO:		
Durango:	$n = 24$	68-19, 68-67, 6293 (GH)
Cosmos pringlei B. L. Robinson		
MEXICO:		
Chihuahua:	$n = 43$-46	6265 (GH)
Durango:	$n =$ ca 48	68-68
Cosmos purpureus (DC.) Hemsl.		
MEXICO:		
Mexico:	$n = 24$	67-149A, 67-149B

TABLE 2. CHROMOSOME COUNTS OF *COSMOS*		
COUNTRY: State	**Taxon**	**Collections**
Cosmos purpureus (DC.) Hemsl. (cont'd)		
MEXICO (cont'd):		
Mexico (cont'd):	*n* = ca 24 (23 + 2 fragments	67-150A, 67-150B
Cosmos scabiosoides H. B. K.		
MEXICO:		
Morelos:	*n* = 12	67-129
Veracruz:	*n* = 12	67-113
Cosmos sessilis Sherff var. *stellatus* (Sherff) Melchert		
MEXICO:		
Michoacán:	*n* = 12	68-123
Cosmos section *Mesinenia:*		
Cosmos carvifolius Benth.		
MEXICO:		
Sinaloa:	*n* = 11	68-24, 68-64, 68-65C
Cosmos carvifolius x *C. linearifolius* F_1 Hybrid		
MEXICO:		
Sinaloa:	*n* = 11	68-65B
Cosmos crithmifolius H. B. K.		
MEXICO:		
Oaxaca:	*n* = 33	67-99, Cruden 1533
	n = ca 33	67-70, 67-85
	n = 33 + 13 dots	67-112

TABLE 2. CHROMOSOME COUNTS OF *COSMOS*		
COUNTRY: **State**	**Taxon**	**Collections**
Cosmos intercedens Sherff		
MEXICO:		
Jalisco:	$n = 11$	68-111, 68-118
Cosmos landii var. *achalconensis* Melchert		
MEXICO:		
Jalisco:	$n = 22$	68-63, 68-121
Cosmos landii var. *landii* Sherff		
MEXICO:		
Jalisco:	$n = 22$	68-61
Cosmos linearifolius (Sch.-Bip.) Hemsl.		
MEXICO:		
Sinaloa:	$n = 11$ (meiosis irregular)	68-65A
Cosmos ochroleucoflorus Melchert		
MEXICO:		
Durango:	$n = 22$	68-22, 68-66, 68-80, 68-86

TABLE 3. CHROMOSOME COUNTS OF *THELESPERMA*		
COUNTRY: **State**	**Taxon**	**Collections**
Thelesperma longipes A. Gray		
MEXICO:		
Nuevo Leon:	$n = 20$	67-5, 67-9, 71-36
Thelesperma megapotamicum (Spreng.) Kuntze		
MEXICO:		
Chihuahua:	$n = 22$	6251
Coahuila:	$n =$ ca 22	71-34
	$2n = 44$	
UNITED STATES:		
New Mexico:	$n = 11$	68-14
Thelesperma simplicifolium var. *macrocarpum* Melchert		
MEXICO:		
Coahuila	$n = 30$	71-33
Nuevo Leon:	$n = 30$	67-1A, 67-1B-, 67-1C, 67-1D, 67-3, 67-12, Cruden 1521
	$n =$ ca 30	67-10

THE TYPE OF *AMSONIA TABERNAEMONTANA* WALTER (APOCYNACEAE): A DISCOURSE ON THE LIMITS OF "INDIRECT REFERENCE"

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ABSTRACT

The argument has been made elsewhere that the type of Thomas Walter's *Amsonia tabernaemontana* (Apocynaceae) is a specimen not seen by Walter but one which has been designated a lectotype now in the Linnaean herbarium, London. The counter-argument, made here, is that Walter did not base his new species on the publication nor specimen of Linnaeus, but rather created a new species whose neotype is held by an institution of the United States. *Phytologia 92(3): 334-344 (December 1, 2010).*

INTRODUCTION

It is understood that for a new plant species to merit scientific recognition it must be given a two-part name and a Latin diagnosis or description. Though in recent decades it is also obligatory that a new species be represented by a type specimen, this stricture did not apply in the early years. For names published prior to 1958, in those cases where a type is missing, the International Code of Botanical Nomenclature (McNeill et al. 2006) permits any person to designate a lectotype from surviving original materials or, if they are absent, to select a neotype from any source as its replacement. It is vital for future nomenclatural stability that the Code be faithfully followed. At times, as here, that obligation is slighted, and the wayward agent must be called to account.

THE BACKGROUND

In the 1780s, Thomas Walter, a rice-plantation owner in South Carolina, published a small book, *Flora Caroliniana* (1788), in which he described 1056 plant species that he observed about him, 404 of them he believed to be new to science (Ward 2007a; 2007b; 2007c; 2008a; 2008b). Many of his names for these new species are in modern use, and the author designation "Walt." is familiar to all students of the southeastern American flora.   One of these names, *Amsonia tabernaemontana* Walt. (Apocynaceae), although the genus, its epithet, and even its author are not in question, has now generated transoceanic dispute as to its type.

Linnaeus (1753), in the first edition of his famous *Species Plantarum*, named and described three species native to the tropics (and themselves not relevant to this discussion).   He chose for them the generic name *Tabernaemontana*, the word commemorating a 16th century German herbalist.   Linnaeus (1762), in his second edition, appreciably expanded the number of species, including one he named *Tabernaemontana Amsonia*. He described it with four words of his own: "*foliis alternis, caulibus subherbaceis.*"   But he followed this quite worthless diagnosis with a rather lengthy (35 word) description taken from the *Flora Virginica* of Gronovius (1739: 26). The names of Gronovius' book, as being "pre-Linnaean" (i.e., pre-1753), carry no nomenclatural standing (but see below).

Gronovius noted *Tabernaemontana Amsonia* to occur in "Virginia."   The larger part of his *Flora* was based on specimens and descriptions sent to him by John Clayton, a tobacco-plantation owner and county clerk of Gloucester County, coastal Virginia (Berkeley & Berkeley 1963).   It was Clayton, on specimens sent by him to Gronovius and other European botanists, who first used the word "*Amsonia.*" [This eponym has been traced (Pringle 2004) to a Dr. John Amson, a physician of nearby Williamsburg, Virginia, and presumed friend of Clayton's.]

It is known (Hitchcock 1905: 32; Ward, in ms.) that Walter possessed and faithfully used a copy of Linnaeus' *Species Plantarum*, 2nd edition. (He apparently never saw the somewhat rarer 1st edition.)

He thus would have been familiar with Linnaeus' treatment of the genus *Tabernaemontana* and Gronovius' descriptive text of *T. Amsonia*. He may have recognized the plant he knew in South Carolina to match Gronovius' description. But Walter chose, when including his plant in his own *Flora* (1788: 98), to treat it in a new genus apart from that of Linnaeus. [In this, modern botanists have given him full support (Mabberley 1996: 34, 697).] He then composed a new diagnosis, transposed the two words of the name given it by Linnaeus, and published his plant under the name *Amsonia Tabernaemontana*. It is conventionally recorded in modern botanical works as *Amsonia tabernaemontana* Walt., and carries the vernacular name "Blue-star."

Walter retained no types. A folio herbarium of 690 small, often fragmentary, specimens collected in the Carolinas and Georgia in 1787 and seen at least in part by Walter has often been thought to be Walter's herbarium. It is not! (Ward 2007d). It is wholly the gatherings of John Fraser, the Scottish horticultural explorer, though a few of Fraser's specimens have been selected as lectotypes and neotypes of Walter's names (Ward 2007b). A specimen in the folio (*5-C, BM*) was labeled by Fraser as "*Amsonia Tabernaemontana*," but there is no indication it was seen or used by Walter. Even so, there is no reason to doubt the plant held by Walter was the *Amsonia tabernaemontana* as understood by modern authors (Woodson 1928; Radford et al. 1968; Ward 2008b). His "*fol. ovatis*" serves to distinguish it from the similar but narrow-leaved *A. tabernaemontana* var. *salicifolia* (Pursh) Woodson. And it is known to occur in the area of South Carolina frequented by Walter.

[In addition to *Amsonia tabernaemontana*, Walter encountered and described a second species, *Amsonia ciliata* Walt. There is no type; a neotype has been selected (Ward 2007c). Though somewhat similar to *A. tabernaemontana*, this second species is uniformly recognized as distinct. Its nomenclature causes no conflict.]

Walter's full diagnosis for *Amsonia tabernaemontana* ("*fol. ovatis utrinque acutis; floribus caeruleis*"), other than indicating the blue flower color, is scarcely useful. It is however quite independent of that of Gronovius. Not a phrase, not a single word, used by Walter appears in the description by Gronovius. [Gronovius did not mention

flower color. And leaf shape was described with different words ("*Folia...lato-lanceolata*").] In the belief that Walter intended and achieved the formation of a new name for the Blue-star by his publication of *Amsonia Tabernaemontana*, and with knowledge there was no extant type, Ward (2008b) selected a neotype: *Smith 1114, 3 June 1941 (USCH)*, from Witherspoon Island, Darlington County, South Carolina.

THE ARGUMENT

But forces of dispute were gathering in Europe. In the 1980s the Natural History Museum, London, began an effort to typify *all* the thousands of names formed by Linnaeus in his detailed publications (Cannon et al. 1983). This enormous task is now near completion, under the stewardship of Charles E. Jarvis and publication of his voluminous *Order out of Chaos* (2007). However, a small scattering of names of North American plants was bypassed, and their typification has now been redressed by Reveal & Jarvis (2009).

Reveal & Jarvis (2009) dealt with 42 names. For each they gave citation of the original Linnaean name, the modern name, and a neotype or lectotype selected insofar as possible from the Linnaean Herbarium, London. Most of their entries are concise, some as brief as four lines, yet fully sufficient for selection of a type. For *Tabernaemontana amsonia* L. (1762) they spoke at length (46 lines). They designated a lectotype, a specimen in the Linnaean Herbarium (*LINN 304.5*), of the Blue-star (though they do not use that name). This specimen consists of a stem with three large leaves and several smaller ones, as well as a several-flowered terminal inflorescence. The sheet bears "*Amsonia*" as well as "Solander" (not visible on microfiche), both words in the hand of Linnaeus (Savage 1945). It is not the specimen (*Clayton 306*) used by Gronovius in the writing of his book; that specimen seems to have been lost.

Reveal & Jarvis considered in some detail the pathway by which Linnaeus may have received the Linnaean Herbarium specimen. They thought it possible that it had been sent by Clayton to Gronovius, then passed on to Joseph Banks, to Daniel Solander, to James Edward Smith, and on to Linnaeus. [This sequence cannot be. Banks did not

acquire the Gronovius herbarium until 1794, after Linnaeus' death in 1778 and long after his 2nd edition in 1762. The probable source is to be found among the Linnaean correspondence (Berkeley & Berkeley 1963: 135). In November 1761, Solander wrote Linnaeus: "All the information I could give relating to *Amsonia* will be seen from the annexed [attached] description made from the living plant which flowered this year...." The specimen (*LINN 304.5*) was thus grown in England and could never have been seen by Clayton. Since this specimen was with Linnaeus before 1762 and thus "original material" (McNeill et al. 2006), it is a suitable choice for designation as lectotype, as was done by Reveal & Jarvis. But, as noted by these authors, their discussion (as is this one) of Linnaeus' source was merely "academic," not serving to advance their argument.]

Reveal & Jarvis (2009) argued that the specimen in the Linnaean Herbarium (*LINN 304.5*) is the type (the lectotype, by their action) of Linnaeus' *Tabernaemontana Amsonia* (1762), as well as Walter's *Amsonia Tabernaemontana* (1788), that the two names are homotypic. They made this linkage through the references they found in *Flora Caroliniana* to Linnaeus and his books. They quoted at length, without translation from the Latin, from Walter's title page and his "*praefatio*" (preface). They stated these Linnaean extracts demonstrated "clearly indirect references" to Linnaeus' works (and, by implication, to his diagnosis and specimen). They did not cite provisions of the Code (Art. 32.5, Art. 52.1) which were perhaps relevant.

Reveal & Jarvis (2009) called attention to the use in Walter's book of *italic* for most new names and roman for existing names. Since Walter's "*Tabernaemontana*" is in roman, and *Amsonia ciliata*, an undisputed Walter name, is in *italic*, they reasoned that Walter intended his *Tabernaemontana* not to be a new name, but that he considered it to be an existing name.

Reveal & Jarvis (2009) concluded their discussion by faulting the statement of Ward (2008b) that, if Walter's type was the same as Linnaeus,' Walter's name would be superfluous and thus illegitimate. The statement of Ward is of course incorrect. Since Walter was creating a new genus apart from *Tabernaemontana*, his circumscription

would be different and thus not trigger rejection by the Code (Art. 52.1).

[Reveal & Jarvis did not comment on the peculiarity of Walter's *Amsonia Tabernaemontana* being a transposition of Linnaeus' *Tabernaemontana Amsonia*. This unique reuse of old words, never elsewhere employed by Walter, has suggested to some (Pringle 2004; J.S.P., pers. comm., Dec 2005, Mar 2010) that the Walter name must have been based on the Linnaean name. If Walter indeed considered his *A. tabernaemontana* and Linnaeus' *T. amsonia* to be the same species, Walter's name would have the same type as did that of Linnaeus. This would be true even though the Solander specimen now treated as Linnaeus' type was unknown to Walter. But Walter was prolific in his formation of new names for species already named by Linnaeus. And he did not indicate a linkage of his name with Linnaeus' by insertion of a marginal note, as he did elsewhere (see below). Thus an effort to reconstruct Walter's intent in 1788 remains conjectural. Since Reveal & Jarvis' argument rests elsewhere, the significance of this transposition is not further addressed here.]

To their credit, Reveal & Jarvis trusted to the experience of their readers to understand that Linnaeus' epithet "*Amsonia*," though prior to Walter's epithet "*Tabernaemontana*," could not under the Code (Art. 23.4) be transferred to Walter's genus *Amsonia*, else forming the prohibited tautonym *Amsonia amsonia*.

THE COUNTER-ARGUMENT

Little need be said in refutation of the argument put forth by Reveal & Jarvis (2009) that the type of *Amsonia Tabernaemontana* Walter (1788) is identical with the type of *Tabernaemontana Amsonia* Linnaeus (1762). When their claims are fully dissected (as above), persons with nomenclatural understanding will see flaws.

These flaws need be put on record. Reveal & Jarvis (2009) relied most centrally on the linkage they saw in Walter's references to Linnaeus. The 36 words (in Latin) from Walter's title page consist largely of description of the included text, in the exuberant style followed by Linnaeus himself (1753, 1762) and other 18th century

writers. The only mention of Linnaeus on the title page lies in the words "*perillustris Linnaei digesta*" or "well illustrated in the order (or system) of Linnaeus." The 40 words quoted from Walter's preface were neatly translated by Rembert (1980): "When the author of this compendium first undertook his botanical inquiries, there was no help for him beyond that which *Systema Naturae* and *Genera et Species Plantarum*, the works of the most distinguished Linnaeus, provided" (page v). And: "He has followed the Principles of the *Systema* of Linnaeus rather than the words, and however often he has neglected the words, he has all the more endeavored to consult the principles" (page vii).

These words of Walter's are indeed references to Linnaeus and his works. The Code permits (Art. 32.5, 32.6) that a reference published before 1953 may be either direct or indirect. An indirect reference must be "a clear (if cryptic) indication by an author citation or in some other way" that a previously published name is considered by the author to be synonymous. The usual purpose of such indirect citation is to retain a name that somehow failed to meet the publication requirements of the Code. (The five examples cited under Art. 32.6 all demonstrate this use.)

But one is hard put to see Walter's bland words of praise for Linnaeus to be more than good manners, showing his profound respect for a deceased giant. (Linnaeus' death preceded Walter's *Flora* by ten years.) There is no mention by Walter of any of the many Linnaean species whose names he had adopted, much less a credit to Linnaeus for species he considered his own discovery. The implication that Walter's language provides "clearly indirect references" to Linnaeus' diagnosis and specimen of *Tabernaemontana Amsonia* is just incorrect.

Another provision of the Code may be a part of Reveal & Jarvis' reasoning. A linkage between a new name and a pre-existing synonymous name at times has caused an author of a new name inadvertently to invalidate his new creation. This is the requirement that a name is to be rejected if it was "nomenclaturally superfluous" when published (Art. 52.1, 52.2), that is, if the author included within its cicumscription an earlier name which by the rules should have been adopted. The circumstances that would cause rejection under this

provision are more narrow than those permitting retention of a name by an "indirect reference." They require, in effect, citation of the pre-existing name; an "indirect reference" would not serve. (Ten supporting examples are given under Art. 52.2.)

It is unclear which, if either, of these provisions of the Code were considered by Reveal & Jarvis to be critical to their argument, or indeed if their basis was some provision overlooked here. They cited no terms of the Code upon which they rely. Neither the provision for retention of an imperfect name, nor the provision for rejection of a superfluous name, seems applicable. Their use of "indirect references" may suggest they were endeavoring to retain Walter's imperfectly published name. But that cannot be, for Walter *does* meet all requirements for valid publication of his new names, confirmation of which is attested by acceptance of his names by all modern authors. [Walter did overstep the tolerance of future nomenclaturalists in one regard. He placed 44 of his species in 28 different genera, all named "*Anonymos*" (Ward 1962; Wilbur 1962). But these names have long since been sanctioned.]

As to Walter's presumed use of *italic* in designating his new species, he made no mention of this practice in his preface (though he did note his use of *italic* for words in his diagnoses that he wished to emphasize). Some 17 new Walter names have been identified that are not italicized: *Ambrosia simplicifolia, Amorpha herbacea, Apium bipinnatum, Arethusa racemosa, Aster carolinianus, Frasera caroliniensis, Hamamelis dioica, Hibiscus coccineus*, etc. These omissions were also noted by Hitchcock (1905: 32). Their numbers open the possibility that they were added by another party. Fraser, idly thumbing the pages of Walter's manuscript during the months-long sea journey back to England, cannot be excluded as their author (Ward, ms.). They cannot be relied on as certain indicators of those species Walter considered as his own.

A significant point was overlooked by Reveal & Jarvis, in that Walter occasionally *did* disagree with the placement of a Linnnaean name. He then made a new combination and marked it as new by inclusion of the Linnaean basionym as a marginal note. Examples: *Ervum volubile*, based on *Hedysarum volubile* L.; *Hedera arborea*,

based on *Vitis arborea* L.; *Origanum incanum*, based on *Clinopodium incanum* L.; *Sophora perfoliata*, based on *Crotalaria perfoliata* L.; etc. About a dozen such new combinations have been noted.

CONCLUSION

Thus, if examination of Walter's title page and preface as cited by Reveal & Jarvis shows no "clear" linkage between Walter's *Amsonia Tabernaemontana* and Linnaeus' *Tabernaemontana Amsonia*, if the use under the Code of "indirect references" is limited to situations where the original publication is defective, if the failure to use *italic* as an indication of Walter's intent not to form a new species is unreliable, and if Walter cited his new combinations in a consistent way different from that postulated, no basis remains for a claim that Walter's type is homotypic with that of Linnaeus. *Amsonia tabernaemontana* Walt., supported by its neotype (USCH), still stands, and *Tabernaemontana Amsonia* L., with its lectotype (LINN), remains its heterotypic synonym.

OVERVIEW

This is clearly a case of the dark forces of the giant phytomorgues of Europe, having by virtue of historic opportunity, possession of the great part of American plant types, now striving to seize title to the types of the few Yankee plants yet outside their grasp. They do so by stealth, secreting their tactical advances by seemingly innocuous documents hidden within obscure and little-read journals of European occult societies. Too soon, should their avaricious advances be unopposed, loyal red-blooded Americans would find themselves shorn of all power to identify the plants necessary to their weal, without paying obeisance to their foreign nomenclatural masters. This intrusion must be resisted unto death. In the vernacular: Keep your cotton-pickin' hands off our Thomas Walter types !!

ACKNOWLEDGMENTS

with his monumental labors in documenting the Linnaean names, have reputations far beyond my skill to sully. But here they have made an error of typification, and I cannot but gloat at having caught them out. I doubt it will ever happen again.

I have been greatly aided in composition of this essay by Robert L. Wilbur and John B. Nelson, and benefited from the wise advice of James S. Pringle. Drs. Reveal and Jarvis continue to receive my full respect and gratitude for their assistance in nomenclatural matters elsewhere.

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BAHIA DEPAUPERATA (ASTERACEAE: BAHIEAE) TRANSFERRED TO ACHYROPAPPUS

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ABSTRACT

Bahia depauperata is transferred to *Achyropappus*. *Phytologia 92(3): 345-345 (December 1, 2010).*

KEY WORDS: *Bahia depauperata, Achyropappus.*

ACHYROPAPPUS DEPAUPERATUS (S.F. Blake) B.L. Turner, **comb. nov.**
Based upon *Bahia depauperata* S.F. Blake, Brittonia 2: 353. 1937.

Blake, in his original description, reckoned the Guatemalan *Bahia depauperata* to be better positioned in the genus *Bahia*, comparing it to *B. anthemoides* (H.B.K.) A. Gray (= *Achyropappus anthemoides* H.B.K.) but Baldwin et al. (2002), using DNA data, retained both genera. Nash and Williams (1976) in their account of Asteraceae for the Flora of Guatemala, failed to account for the taxon concerned.

In short, *Bahia depauperata* seems best positioned in the genus *Achyropappus*, along with its closest relative, *A. anthemoides*, a relationship also suggested by Blake.

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DNA FROM HERBARIUM SPECIMENS: I. CORRELATION OF DNA SIZE WITH SPECIMEN AGE

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ABSTRACT

Comparisons are made of DNA [(mode, bp); range (max, min)] extracted from 50 herbarium specimens of *Juniperus* ranging from 1 to 80 years old. The size of DNA declined with age, but varied considerably for specimens less than 20 yrs. old. After about 20 yr. the size of the DNA appeared to asymptote at about 200 - 500 bp. Variation in the quality of DNA from recent specimens may be due to drying methods and storage conditions (humidity, temperature). Degradation in older specimens may be more influenced by oxidative processes. *Phytologia 92(3): 346-353 (December 1, 2010).*

KEY WORDS: DNA, herbarium specimens, degradation, *Juniperus*.

It is now considered standard procedure to collect specimens and put some leaves in silica gel for subsequent DNA extraction. Liston et al. (1990) seem to be the first to utilize silica gel in the field, although Doyle and Doyle (1987) suggested that drying appears to be effective in preserving DNA and Doyle and Dickson (1987) also found that drying was a good preservation method for DNA restriction analysis.. Later, Chase and Hills (1991) promoted silica gel for field preservation. However, with the ephemeral condition of some species, the increased restrictions on the transport of plant materials and the geopolitical problems encountered in collecting, obtaining DNA from herbarium materials is sometimes the only option.

In a seminal paper, Rogers and Bendich (1985) reported on the extraction of DNA, using the CTAB method, from herbarium and mummified plant materials. They concluded that the condition of the leaf DNA depended on the method of drying, and developmental stage. They obtained DNA that could be restricted from dried specimens ranging from 22 - 118 yr. old and mummified seeds up to 44K years old.

But, Nickrent (1994) cited problems in obtaining good DNA from specimens due to age and drying procedures as well as the use of chemicals such, as ethanol and formaldehyde, to combat fungal growth in moist, tropical areas.

Drabkova et al. (2002) compared 7 methods for DNA extraction from herbarium specimens of the Juncaceae and concluded that the Qiagen DNeasy Plant Kit and hot CTAB protocol generally gave the best results. They noted that air-dried specimens (up to 42ºC) generally gave suitable DNA.

However, the drying methods utilized in the past were subject to considerable variation among institutions. The purpose of this study is to establish some baseline values for DNA quality from *Juniperus* specimens of various ages.

MATERIALS AND METHODS

Plant herbarium specimens utilized:

BAYLU - *Juniperus virginiana* L., *Adams 2433*, 1977; *J. scopulorum* Sarg., *Adams 2512*, 1978. *J. coahuilensis* (Mart.) Gaussen ex R. P. Adams, *Adams 387*, 1970, *Adams 438*, 1970, *Adams 1474*, 1975, *Adams 1511*, 1975, *Adams 2522*, 1978, *Adams 2539*, 1978, *Adams 6829*, 1991, *Adams 10634*, 2005, *Adams 11849*, 2009, *J. ashei var. ovata* R. P. Adams, *Adams 98*, 1970,

OSC - *J. communis*, *OSC100300*, 1957, *OSC97657*, 1957, *OSC152071*, 1979, *OSC210646*, 1997, *OSC25127*, 1930, *OSC103501*, 1955, *OSC110375,* 1979, *OSC91155*, 1953, *OSC116403*, 1962, *OSC111301*, 1932, *OSC194044*, 1995, *OSC05533*, 1951, *OSC150461*, 1977,

OSC70268, 1948, *OSC70142*, 1947, *OSC100299*, 1957, *OSC97972*, 1955.

VPI - *J. virginiana*, *VPI 105320, Wright 8992*, 1999, *VPI105305, Wright 7730*, 1997, *VPI105314, Wright 7731*, 1997, *VPI105312, Wright 8745*, 1999, *VPI 105319, Wright 8986*, 1999, *VPI105309, Wright 8119*, 1998, *VPI105313, Wright 8120*, 1998, *VPI105317, Wright 8988, 1999, VPI105318*, Wright 7290, 1999, *VPI105315, Wright 7291, 1999, VPI105316, Wright 8917, 1999, VPI105310, Wright 7815*, 1997, *VPI105311, Wright 8012*, 1997, *VPI105308, Wright 7951*, 1997, *VPI105306, Wright 7947*, 1997, *VPI103759, Townsend 3702*, 2006, *VPI103702, Townsend 3710*, 2006, *VPI103703,* Townsend 3712, 2006, *VPI100283, Perkinson ns,* 2000, *VPI35799, Connell ns,* 1947, *VPI51546, Crutchfield 5604*, 1967, *VPI69443, Godfrey 70154*, 1971.

DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions. Genomic DNA was visualized by agarose gel electrophoresis by mixing 3 µl DNA extract, 3 µl pGEM markers and 3 µl λ\HindIII, and loading 6 µl on a 0.6% agarose gel, then running at 100 v for 20 min.   The images were captured on a Kodak Gel Logic 100 Imaging System, and profile analysis was used to determine the modal DNA size and range of DNA sizes.   The DNA from some samples was subjected to PCR amplification.   ITS (nrDNA) and petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

RESULTS

Table 1 shows the sizes of DNAs and ranges for the 50 herbarium specimens analyzed. It seems unusual that even the most recent specimens (2009, 1 yr. old) had some degraded DNA, but this may be due to normal shearing during leaf grinding with a mortar and

pestle. The linear correlation between specimen ages and DNA modal sizes is -0.58 (33.6% of the variance). Graphing specimen ages vs. modal sizes is shown in Figure 1. Notice that a quasi-logarithmic curve shows that the trend is for the DNA sizes to rapidly decline to about 200-500 bp after 30 years. Paabo (1989) found little correlation between DNA sizes and ages in mummified animal samples. He found remarkably uniform genomic degraded DNAs from samples ranging in age from 4 to 5,000 years old (~70 bp - 500 bp, with a mode of ~200-400 bp).
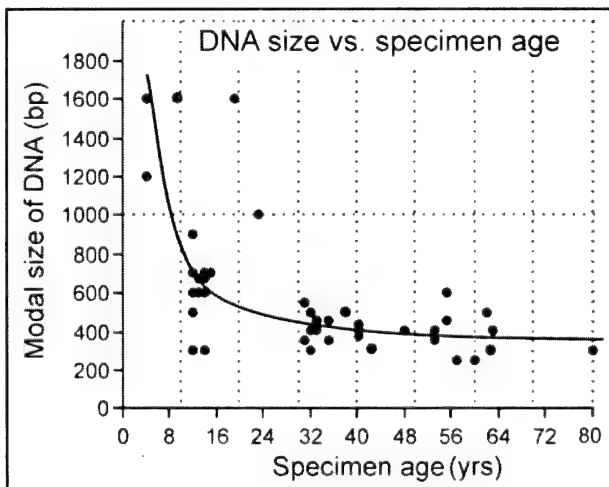
Figure 1. Plot of modal size of DNA vs. specimen age in years.

There are a number of outliers (Figure 1) that have much larger DNA sizes than average. It may be that these DNAs are contaminated with modern fungal/ and or bacterial DNA. Additional testing is being conducted to determine this. There are also a number of samples that have unusually degraded DNA from relative new specimens (Fig. 1). A large amount of variation in DNA sizes is particularly noticeable during the first 20 years (Fig. 1), after which, the sizes are much more uniform.

Several specimens of *Juniperus*, collected by the senior author and dried and stored in comparable conditions, provide a useful serial view of DNA degradation (Fig. 2). Profile analyses of progressively older specimens shows (Fig. 2) that large amounts of degradation are
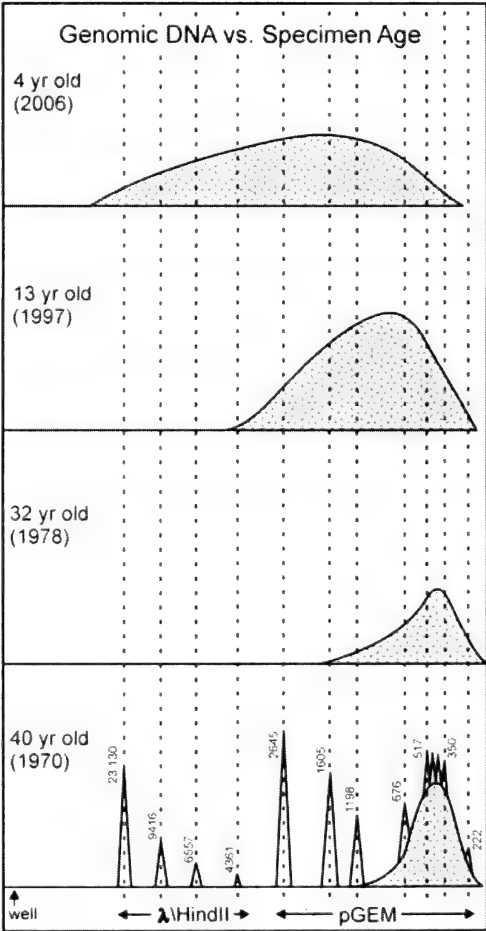
Figure 2. Comparison of genomic DNAs from 4, 13, 32, and 40 year old *Juniperus* specimens.

accumulated during the first 4 years and that the amounts of large DNA fragments (100K - 1000 bp) gradually declines with age in this set of specimens. Because these data are enhanced by the analysis of more concentrated extracts (to be able to see the DNA), it is not apparent from this series, but we have observed that both quantity and quality declines as specimens age. However, the decline in DNA yields is generally not as much of a concern as the quality, as nearly all studies utilize PCR to amplify gene regions of interest, and only minute amounts of DNA are needed for PCR.

Paabo (1989) concluded "the rapidity with which the body [*human in this case*] has been desiccated immediately after death is a major factor that determines the extent of size reduction of the DNA." Paabo (1989) further concluded that after initial desiccation, the DNA is severely damaged by oxidation. Although it is relatively easy, today, to curate herbarium specimens in low humdities by air conditioning, there does not seem to be a practical way to store herbarium specimens in an oxygen-free environment. It may be, that due to the large amounts of anti-oxidants in some plant tissues, oxidative degradation of DNA may not be as severe as in human tissue. Additional research is in progress to attempt to address the impact of oxidation on DNA size in herbarium specimens.

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Table 1. Size (modal value) and range of DNA extracted from 50 herbarium specimens. The 24K bp values were assigned to the genomic band that co-elutes with the 23,130 bp λ\HindIII band. These genomic bands likely range from >100,000 to about 20,000 bp (see Fig. 2), but are merely noted as 24K.

Specimen	Date	Mode(bp)	Range (bp)
BAYLU98	1970	430	1100-100
BAYLU387	1970	400	1000-100
BAYLU438	1970	370	1200-100
BAYLU1474	1975	350	1600-100
BAYLU1511	1975	450	1200-100
BAYLU2433	1977	400	9000-100
BAYLU2512	1978	300	2600-100
BAYLU2522	1978	500	1000-100
BAYLU2539	1978	400	1600-100
BAYLU6829	1991	1600	9400-150
BAYLU10634	2005	24K	24K-9400
BAYLU11849	2009	24K	24K-9400
OSU100300	1957	400	2700-100

Table 1. Size (modal value) and range of DNA (continued).

Specimen	Date	Mode(bp)	Range (bp)
OSU97657	1957	400	2600-100
OSU152071	1979	550	2645-150
OSU210646	1997	1000	2700-150
OSU25127	1930	300	600-100
OSU103501	1955	600	2700-150
OSU110375	1979	350	1600-100
OSU91155	1953	250	2600-100
OSU116403	1962	400	2600-100
OSU194044	1995	700	4300-150
OSU05533	1951	250	600-100
OSU150461	1977	450	4400-100
OSU70268	1948	500	800-100
OSU70142	1947	400	1600-100
OSU100299	1957	350	1000-100
OSU97972	1955	450	1200-100
VPI105320	1999	900	24K-150
VPI105305	1997	670	9500-150
VPI105314	1997	300	2600-100
VPI105312	1999	300	6500-150
VPI105319	1999	700	24K-150
VPI105309	1998	600	24K-150
VPI105313	1998	670	24K-150
VPI105317	1999	700	24K-150
VPI105318	1999	500	2600-100
VPI105315	1999	500	4300-100
VPI105316	1999	600	24K-100
VPI105310	1997	700	24K-150
VPI105311	1997	600	4400-150
VPI105308	1997	600	4300-150
VPI105306	1997	600	4300-150
VPI103759	2006	1200	24K-150
VPI103702	2006	1600	24K-150
VPI103703	2006	1600	24K-150
VPI100283	2000	1600	24K-150
VPI35799	1947	300	1600-100
VPI51546	1967	300	2600-100
VPI69443	1971	500	2600-100

A NEW SPECIES OF *EUPATORIASTRUM* (ASTERACEAE: EUPATORIEAE) FROM SAN ANDRÉS TUXTLA, VERACRUZ, MEXICO

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ABSTRACT

Description of **Eupatoriastrum johnbeamanii sp. nov.**, a remarkably distinct novelty from Veracruz, Mexico, brings to seven the number of species found in the genus, well-known for possessing chaff on the receptacle, presumably a relictual trait in the tribe Eupatorieae. A photograph of the holotype is provided. *Phytologia 92(3): 354-357 (December 1, 2010)*.

KEY WORDS: Asteraceae, Eupatorieae, *Eupatoriastrum*, Mexico, Veracruz

Routine identification of Mexican comps has revealed the following novelty:

√ **EUPATORIASTRUM JOHNBEAMANII** B.L. Turner, **sp. nov.**
Fig. 1
    Eupatoriastrum nelsonii Greenm. similis sed differt foliis longioribus (20-35 cm vs 10-20 cm) penninervatis (vs. 3-5-nervatis ad basim) lineari-lanceolatis latissimis prope medium (vs. late ovatis latissimis prope basim).

    TYPE: **MEXICO. VERACRUZ: Mpio. San Andrés Tuxtla**, "Catemaco. Mapa 21.0/57.5, 2 km al E de Lago Catemaco en el camino al Bastonal," 540 m, 10 May 1972, *John H. Beaman 5911* (holotype: MSC).

Suffruticose perennial herbs or shrublets 1.0–2.5 m high. **Stems** to 1 cm thick, pubescent with short stout, spreading, trichomes, the vestiture ca 0.5 mm high. **Leaves** very large (20–35 cm long, 6–10 cm across), alternate, penninervate, linear-lanceolate, widest at or near the middle, essentially glabrous above and below, except along the major veins; petioles 1–4 cm long, grading into the blades, the margins entire below, weakly serrate above. **Capitulescence** a large (many-headed), terminal, cymose panicle, the ultimate peduncles 1–3 cm long. **Heads** broadly campanulate, ca 12 mm high, 15 mm across. **Receptacles** convex, glabrous, ca 3 mm across, paleate throughout. **Involucral bracts** 6-8 seriate, imbricate, the innermost linear-lanceolate, scarious, glabrous, the apices rounded, grading into the palea. **Florets** numerous (100+); corollas yellow, ca 6 mm long, glabrous or nearly so; tubes ca 3 mm long, the lobes ca 0.5 mm long, ornate with golden glandular globules. **Anther appendages** narrowly triangular, ca 0.5 mm long. **Achenes** black, weakly 3–5 sided, sparsely pubescent, the podia well-developed; pappus of numerous bristles 5–6 mm long.

ADDITIONAL COLLECTIONS: **MEXICO. VERACRUZ: Mpio. San Andres Tuxtla,** "Mapa 23.0/55.0, lado W de Cerro Mastagaga ca. 13 km al N.E. de San Andrés Tuxtla," 1200 m, 29 Jan 1972, *Beaman 5553* (MSC, 2 sheets).

Other than features of leaf morphology, as noted in the above diagnosis, *Eupatoriastrum johnbeamanii* differs from *E. nelsonii* in numerous other characters, including capitulescence (many-headed, terminal panicles vs. few-headed, axillary cymes), involucres 6–8 seriate (vs 3–5 seriate), and yet other features of the capitula.

Eupatoriastrum is a relatively rare genus confined largely to Mexico. Turner (1997) provided an account of the five species known to him at the time, four of these illustrated with full-page figures. An additional species was subsequently added to the genus (Turner 2008); the present novelty brings the total to seven.

The species is named for my long-time friend and colleague John Beaman. We both worked at Washington State University under the tutelage of the late Marion Ownbey at the same time (he for a

masters; me for a doctorate). John went on to obtain his doctorate under Reed Rollins at Harvard and has become widely known for his extraordinary botanical studies on Mount Kinabalu, largely working out of KEW. I have used his first name in my eponym so as to distinguish him from his son Reed, who also is widely known for his contributions to Asian Botany.

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Fig. 1. *Eupatoriastrum johnbeamanii* (holotype: MSC).

SMALLANTHUS PUTLANUS (ASTERACEAE: HELIANTHEAE), A NEW SPECIES FROM OAXACA, MEXICO

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ABSTRACT

A new species, **Smallanthus putlanus** B.L. Turner, is described from westernmost Oaxaca. It is seemingly most closely similar to *S. macvaughii* of Jalisco but can be recognized by several features. A photograph of the type specimen is provided, along with maps showing the distribution of the Mexican taxa of *Smallanthus*. *Phytologia 92(3): 358-361 (December 1, 2010).*

KEY WORDS: Asteraceae, Heliantheae, *Smallanthus*, Oaxaca, Mexico

Routine identification of Mexican Asteraceae has revealed the following novelty:

SMALLANTHUS PUTLANUS B.L. Turner, **sp. nov. Fig. 1**
          Smallantho macvaughii (J. Wells) H. Rob. similis sed differt statura minore (0.5–1.5 m in altitudine vs 2–5 m), bracteis exterior involucralibus minoribus 5–8 mm in longitudine sparsim glandularibus non reflexis (vs 10–20 mm in longitudine dense glandularibus reflexis), et flosculis radii 10–12 (vs. 8).

TYPE: MEXICO. OAXACA: Distrito Putla, Mpio. Santa Cruz Itundujia, " a 0.5 km en LR (W) se la Agencia de la Victoria Bosque de pino-encino secundario." ca 1091 m, 16 44.7 N, 97 45 36 W, 2 Aug 2008, *K. V. Gutierrez 3099*. [con M Trujillo y H. Cruz] (Holotype: TEX).

Perennial herbs, 0.5-1.5 m high. **Stems** villous, arising from woody, corm-like structures, out of which are produced lateral ligneous rootstocks with villous hairs. **Leaves** thin, opposite, 25-30 cm long, 12-14 cm wide; lower surfaces of blades glabrous, hastately to pinnately lobed, the margins finely ciliate; petioles 5-6 cm long, winged throughout, ca 1 cm wide, auriculate at base. **Capitulescence** a terminal, 2-headed raceme, the ultimate peduncles glandular-pubescent, 3-4 cm long. **Heads** ca 3 cm across the extended rays; chaff scarious, linear-lanceolate, slightly shorter than the disk florets. **Outer involucral bracts** 5, narrowly ovate, 5-8 mm long, 2-4 mm wide. **Ray florets**10-12, pistillate, fertile, the ligules yellow, villous at the very base. **Disk florets** sterile (?), 30-40, yellow, sparsely pubescent. **Achenes** of ray florets fertile, ovoid or tear-shaped, glabrous.

Leaf texture and pubescence of *S. putlanus* is especially like that of *S. macvaughii.* When first discerned, I took the present novelty to be a species of the small, mostly herbaceous, genus *Axiniphyllum* (Turner 1978), largely because the type itself was only ca 0.5 cm high. The label data, however, give its height as 1.5 m, more like that of a *Rumfordia*, or *Smallanthus.* In my evaluation of its generic position, the defining characters for *S. putlanus,* were the seemingly rounded, or tear shaped, ray achenes and sterile disk florets, instead of 4-sided and fertile, as found in *Axiniphyllum*, and *Rumfordia*.

Geographically speaking, *Smallanthus putlanus* might also be compared with *S. oaxacanus* (Sch.-Bip.) ex Klatt) H. Rob., an orange-rayed shrubby species of more eastern Oaxaca (Turner 1988). The distribution of the Mexican species of *Smallanthus* is shown in Figs 2-3.

The name is derived from the Distrito de Putla, Oax, whence the type.

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Fig. 1. *Smallanthus putlanus* (holotype).
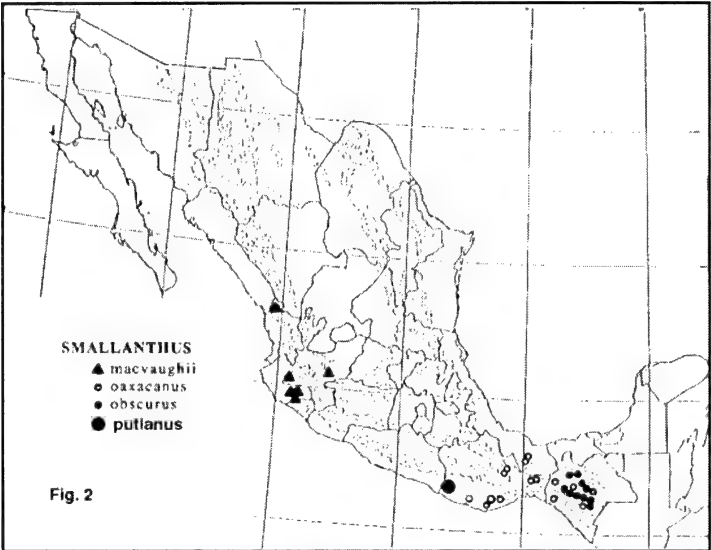
Figure 2. Distribution of Smallanthus species in Mexico.
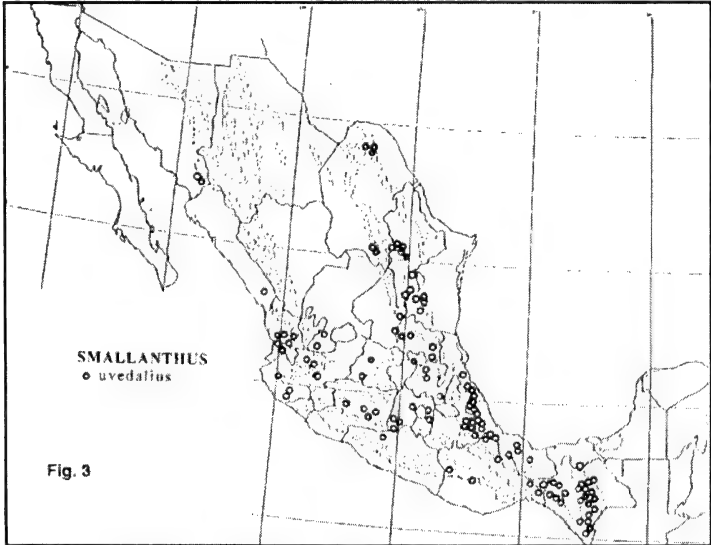
Figure 3. Distribution of *Smallanthus uvedalius* in Mexico.

AGERATINA VILLARREALII (ASTERACEAE: EUPATORIEAE), A NEW SPECIES FROM SIERRA DE ZAPALINAME, COAHUILA, MEXICO

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ABSTRACT

A new species from Coahuila, Mexico, is described as *Ageratina villarrealii*. *Phytologia 92(3): 362-365 (December 1, 2010).*

KEY WORDS: *Ageratina villarrealii,* Asteraceae.

Routine identification of Mexican Asteraceae has revealed the following novelty:

AGERATINA VILLARREALII B.L. Turner, **sp. nov.,** Fig. 1.

Ageratinae parayanae (Espinosa) B.L. Turner similis sed differt plantis minoribus caulibus sparsim puberulis vel glabratis (vs. dense glandulipubescentibus) et foliis late ovatis (vs. cordatis vel subdeltoideis).

TYPE: **MEXICO. COAHUILA: Mpio. de Saltillo**, "Area protegida, Sierra de Zapaliname, el Penitente," ca 25.3495° N, 100. 9049° W, "Bosque de Oyamel de *Psdeudotsuga menziesii* y *Abies vejari*," 29 Sep 2007, *Sergio G. Gomez P. 553* (holotype: TEX).

Perennial herbs to 1 m (?) high. **Stems** sparsely puberulent to glabrate. **Leaves** exceptionally large, 10–12 cm long, 7–8 cm wide, the blades broadly ovate, crenulodentate, palmately nervate from the base, glabrous on both surfaces or nearly so; petioles ca 3 cm long, pubescent like the stems. **Capitulescence** a terminal, congested, cymose panicle ca 6 cm high, 6 cm across, the ultimate peduncles 5–10 mm long, densely pubescent with both glandular and non-glandular

hairs, the vestiture ca 0.5 mm high. **Involucres** ca 6 mm high, the bracts about equal in 2–3 series, sparsely pubescent to nearly glabrous. **Receptacle** ca 1.5 mm across, glabrous. **Corollas** ca 4 mm long; tube glabrous, ca 2 mm long; throat abruptly goblet-shaped, ca 2 mm long, the 5 lobes pilose. **Achenes** 2.5–3.0 mm long, sparsely pubescent; pappus of ca 40 fragile bristles 4–5 mm long.

In my treatment of *Ageratina* in the Comps of Mexico (Turner 1997), this species will key to, or near, *A. parayana*, a species of more southern Mexico, as shown in Fig. 2. The new species is a smaller plant with sparsely puberulent to glabrate stems (vs densely glandular-pubescent) and broadly ovate leaves (vs cordate to subdeltoid).

The novelty was called to my attention almost immediately by the label name "*Flyriella leonensis* (Rob.) K. & H. Rob.," this provided by José A. Villarreal Q. of ANSM, whom the eponym commemorates. Without additional ado, I promptly proposed the name *A. zapalinama* for the taxon, having forgotten that I had earlier proposed such a name for yet another species of *Ageratina* from the same locality (Turner 1991). Regardless, I am pleased to name the present novelty for my colleague José, for he has been a moving force in the field of plant systematics in northern Mexico and elsewhere.

ACKNOWLEDGEMENTS

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Turner, B.L. 1991. Two new species of *Ageratina* (Asteraceae-Eupatorieae) from northeastern Mexico. Phytologia 71: 311–314.
Turner, B.L. 1997. The Comps of Mexico, Vol. 1: Eupatorieae. Phytologia Memoirs 11: 10–50.

Fig. 1. *Ageratina villarrealii* (holotype).
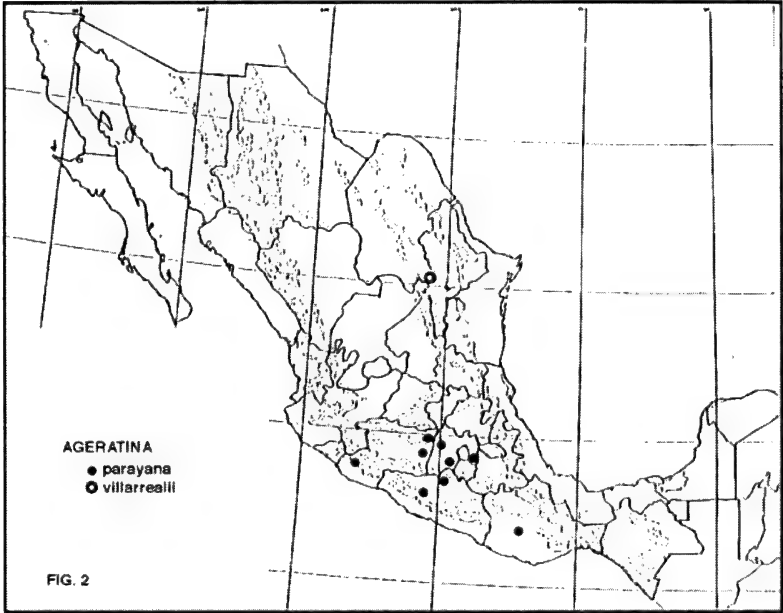
Fig. 2. Distribution of *A. parayana* and *A. villarrealii*.

GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *HESPEROCYPARIS ARIZONICA* AND *H. GLABRA*

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ABSTRACT

The leaf essential oils were analyzed from four *Hesperocyparis* (=*Cupressus*) *arizonica* and five *H. glabra* populations. The leaf oil of *H. arizonica* has large amounts of umbellulone (18.8%), terpinen-4-ol (11.0%), nezukol (11.6%), limonene (6.6%) and β-phellandrene (6.6%) with moderate amounts of α-pinene (4.1%), sabinene (5.3%) and isophyllocladene (3.1%). The oil of *H. glabra* is dominated by cis-muurola-4(14),5-diene (14.3%), umbellulone (9.3%), α-pinene (8.1%), with moderate amounts of limonene (5.6%), β-phellandrene (5.5%), cis-muurola-3,5-diene (5.3%), cis-muurol-5-en-4-one (4.8%), sabinene (4.0%), epi-zonarene (4.0%) and α-acorenol (3.0%). The concentrations of a number of compounds separate *H. arizonica* and *H. glabra*: umbellulone, terpinen-4-ol, 2-ethyl-isomenthone, cis-muurola-3,5-diene, cis-muurola-4(14),5-diene, epi-zonarene, α-alaskene, γ-cadinene, trans-calamenene, δ-cadinene, italicene ether, cis-muurola-5-en-4-α-ol, cis-muurola-5-en-4-α-ol, 3-oxobutyl-isomenthone, α-acorenol, β-acorenol, cadalene, cis-14-nor-muurol-5-en-4-one, oplopanonyl acetate,

isohibaene, isophyllocladene, manoyl oxide, kaur-16-ene and nezukol. Two chemotypes were found in *H. arizonica*: low and high in muurola type compounds. These analyses support the continued recognition of these taxa at the specific level. *Phytologia 92(3): 366-387 (December 1, 2010).*

KEY WORDS: *Hesperocyparis* (=*Cupressus*) *arizonica, H. glabra*, terpenoids, geographic variation, taxonomy.

In the latest nomenclature of the cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere cypresses (exclusive of *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*) and Bartel made the new combinations of *Hesperocyparis arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel. Analyses using RAPDs fingerprinting (Bartel et al., 2003) showed *H. glabra* to be distinct from *H. arizonica* (Fig. 1).
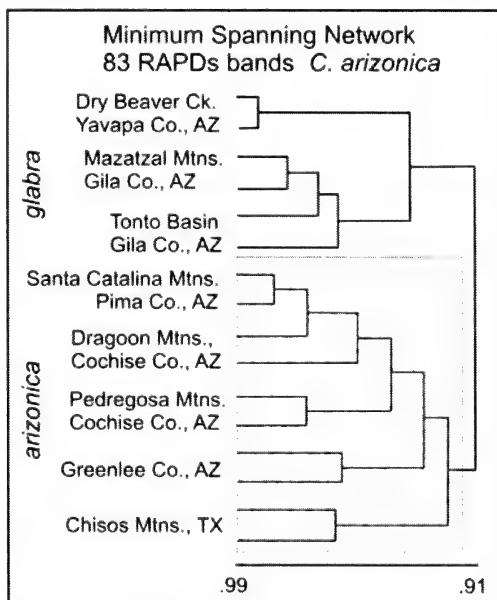
Figure 1. Minimum spanning network (from Bartel et al., 2003).

Contouring the RAPDs clustering of the populations revealed the geographic disjunction between *H. arizonica* and *H. glabra* (Fig. 2).
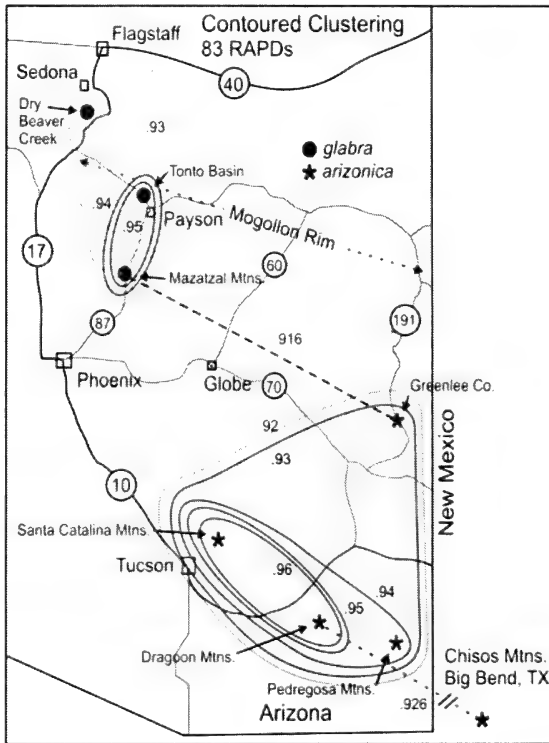
Figure 2. Contoured clustering of populations of *H. arizonica* and *H. glabra* based on 83 RAPDs bands.

The distributions (based on Bartel, 1993) of *H. arizonica* and *H. glabra* are shown in figure 3. Notice the taxa appear to be allopatric except for the new putative population of *H. arizonica* near Prescott. The Prescott collection, if proven to be *H. arizonica,* would be a nearly 200-mile range extension for the species and a significant departure from what was believed to be contrasting habitats and ranges for the two Arizona species.   According to Brown's (1982) map of Biogeographic Provinces (BP) of the Southwest, *H. glabra* is restricted to the Interior (Arizonan) BP (which is largely below the Mogollon

Rim), while *H. arizonica* is found within the "Sky Islands" of the Madrean BP. The Madrean BP, which occurs throughout much of north-central Mexico, only enters the US in southeastern Arizona and extreme southwestern New Mexico. Wolf (1948), Schoenike et al. (1975), Little (2005), Rehfeldt (1997) and other authors have all concluded that *H. arizonica* does not range north of Greenlee County nor west of Pima County.
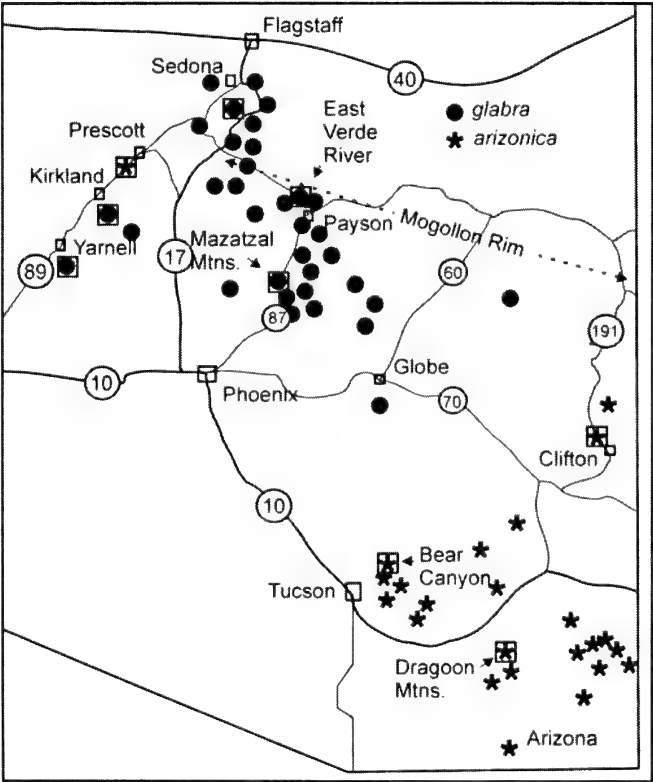
Figure 3. The distributions of the *H. arizonica and H. glabra* (modified from Bartel, 1993). The populations of sampled in this study shown with a box around the symbol.

The leaf essential oil of *H. arizonica* (*C. arizonica*) has been very widely analyzed and reported from mostly cultivated plants (see Emami et al. 2010, for a recent review).

This paper presents the leaf oil compositions and analyses of geographical variation of *H. arizonica* and *H. glabra*.

MATERIALS AND METHODS

Five trees were sampled from four *H. arizonica* and five *H. glabra* populations from locations shown in Figure 3. Collection site information for samples utilized in this study: *Hesperocyparis arizonica*: *Adams 11665-11669*. upper Bear Canyon, 11.8 mi n of Houghton Rd along Catalina Hwy, N 32° 21.801', W 110° 42.765', 1695m, Santa Catalina Mtns., Pima Co., AZ; *Adams 11670-11674*, n side of US191 in dry creek bed, 13 mi. n of Clifton. N 33° 08.057', W 109° 22.608', 1680m, Greenlee Co., AZ, *Adams 11675-11679*, Stronghold Canyon East, 8.5 mi w of US 191, along Ironwood Rd., N 31° 55.540', W 109° 58.007', 1501m, Dragoon Mtns., Cochise Co., AZ; *Adams 12078-12082*, *12301-12310*, 10 mi. sw of Prescott, on Hwy 89, N 34° 27.285', W 112° 32.363', 1657m, Yavapai Co., AZ.

Hesperocyparis glabra, *Adams 11680-11684*, upper Slate Creek, 7.1 mi sw of SR 188, along SR87, N 33° 57.472', W 111° 24.419', 1014m, Mazatzal Mtns., Gila Co., AZ, *Adams 11686-11689*, se of Tonto Natural Bridge St. Park, along SR87, nw of East Verde River, N 34° 18.976', W 111° 23.217', 1475m, Gila Co., AZ, *Adams 11690-11694*, upper Dry Beaver Creek, 0.1 mi. e of SR 179 along Wild Horse Mesa Rd., N 34° 46.131', W 111° 45.779', 1197m, Yavapai Co., AZ, *Adams 12073-12077*, 11 mi. se of Kirkland Jct., Milk Creek, above road crossing, N 34° 18.029', W 112° 29.7096', 1193m, Yavapai Co., AZ, *Adams 12083-12087*, 6 mi. se of Yarnell, AZ, southern Weaver Mtns., N 34° 10.450', W 112° 39.139, 1364m, Yavapai Co., AZ.   All specimens are deposited in the BAYLU herbarium.

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the

samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see 5 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Table 1 shows the composition of average values of *H. arizonica* and *H. glabra,* as well as some plants with unusual oils. The leaf oil of *H. arizonica* has large amounts of umbellulone (18.8%), terpinen-4-ol (11.0%), nezukol (11.6%), limonene (6.6%) and β-phellandrene (6.6%) with moderate amounts of α-pinene (4.1%), sabinene (5.3%) and isophyllocladene (3.1%). The oil of *H. glabra* is dominated by cis-muurola-4(14),5-diene (14.3%), umbellulone (9.3%), α-pinene (8.1%), with moderate amounts of limonene (5.6%), β-phellandrene (5.5%), cis-muurola-3,5-diene (5.3%), cis-muurol-5-en-4-one (4.8%), sabinene (4.0%), epi-zonarene (4.0%) and α-acorenol (3.0%). The concentrations of a number of compounds separate *H. arizonica* and *H. glabra* (Table 1). Particularly useful are umbellulone, terpinen-4-ol,   2-ethyl-isomenthone,   cis-muurola-3,5-diene,   cis-muurola-4(14),5-diene, epi-zonarene, α-alaskene, γ-cadinene, trans-calamenene, δ-cadinene, italicene ether, cis-muurola-5-en-4-α-ol, cis-muurola-5-en-4-α-ol, 3-oxobutyl-isomenthone, α-acorenol, β-acorenol,

cadalene, cis-14-nor-muurol-5-en-4-one, oplopanonyl acetate, isohibaene, isophyllocladene, manoyl oxide, kaur-16-ene and nezukol.

The presence of the murrol family of compounds seems characteristic of *H. glabra* (as opposed to *H. arizonica*). It now appears that samples ascribed to *H. arizonica* (*C. arizonica*) by Adams et al. (1997) were actually *H. glabra*. Likewise, Emami et al. (2010) reported that the leaf oil of *C. arizonica* cultivated in Iran contained all of the muurol components found in *H. glabra*. It seems likely that many or most of the reports on the oil of Arizona cypress cultivated around the world are actually based on *H. glabra*. This observation corroborates Wolf's (1948) assertion that most of the trees cultivated around the world as Arizona cypress are *H. glabra* grown from seed originally collected from the Rye Creek area of Gila County. Incorrectly identified as *Cupressus arizonica* since its introduction into England as early as 1888 (Peattie, 1953), many if not all named Arizona cypress cultivars are derived from *H. glabra* (Jacobson, 1996). Similarly, Posey and Goggans (1967) concluded that the Arizona cypress grown as Christmas trees in the southeastern US likely came from a few individual *H. glabra* trees.

To better visualize the variation among individuals, 63 terpenoids were used to compute similarities among the 46 plant oils and the matrix was factored. This produced eigenroots that accounted for 38.2%, 5.5%, 4.5%, 4.2% and 3.6% of the variance among 46 individuals. Clearly, most of the variance was in the first eigenroot, implying two groups among the data set. A Principal Coordinates Ordination (PCO) divides the 46 individuals into *H. arizonica* and *H. glabra* (Fig. 4). Notice some variation among the *H. glabra* individuals with 2 plants from the Mazatzal Mtns. population loosely clustering, as well as the plants from the Yarnell population.

Based on the position of M1 (11680) on the PCO (Fig. 4), one might suspect that it might be introgressed by *H. arizonica*. However, a close examination of the oil composition (Table 1) reveals that M1 does not contain compounds characteristic of *H. arizonica*, but instead, M1 has very unusual amounts of some compounds (sabinene, limonene, β-phellandrene, citronellol). Moreover, M1 contains the typical muurol components of *H. glabra*.

The oils of the plants in Yarnell population cluster high on axis 3 (Fig. 4). The leaf oil composition of the most extreme plant (Y3, 12085) is shown in Table 1. Y3 is in contrast to M1 in having very low amounts of sabinene, limonene, β-phellandrene but a large amount of nezukol (14.8%) as well as the typical murrol constituents. However, it also contains some components typical of *H. arizonica*: isohibaene and 13-epi-manoyl oxide.
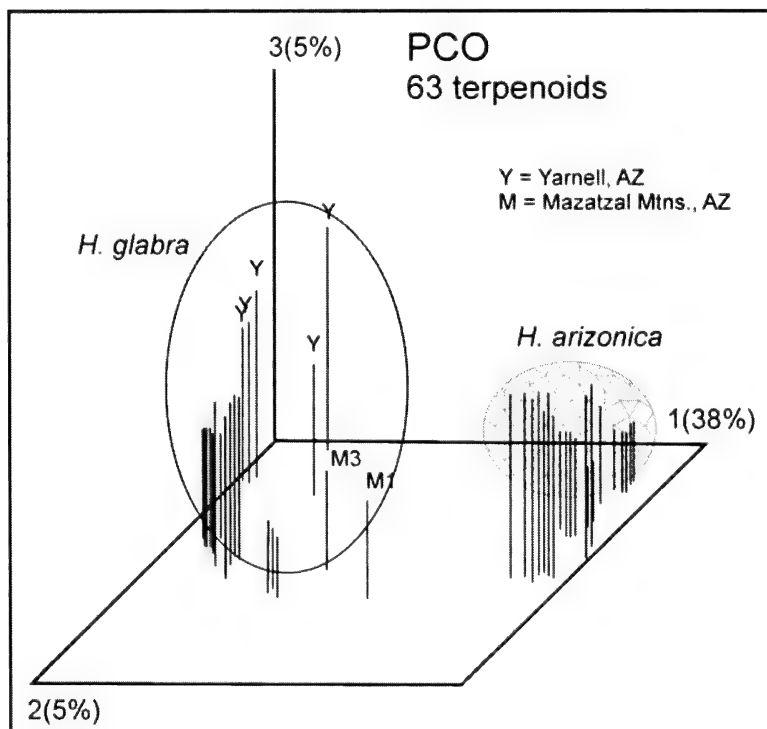

Figure 4. PCO based on 63 terpenoids shows the two major groups: *H. arizonica* and *H. glabra*. M1 (11680) and M3 (11682) are from the Mazatzal Mtns. population and those labeled Y are from the Yarnell population.

Factoring the matrix of the *H. arizonica* similarities resulted in three eigenroots that appear to be biologically significant. These eigenroots accounted for 16%, 12% and 8% of the variance among the 25 samples. Ordination reveals (Fig. 5) that both the Dragoon Mtns. and Prescott populations are di-morphic with individuals that have the muurola related compounds (cis-muurola-3,5-diene, cis-muurola-4(14),5-diene, epi-zonarene, trans-calamenene, cis-muurola-5-en-4-α-ol, cis-muurola-5-en-4-α-ol, and cis-14-nor-muurol-5-en-4-one). This is also seen in table 1 by comparing the *H. arizonica* (ariz) average values with D3 (Dragoon Mtns., *Adams 11677*) and Prescott P2 (*Adams 12079*). In fact, the most similar oil to P2 is D3. Whereas only 2 of 10 trees in the Prescott population had the muurola suite, 3 of 5 trees in
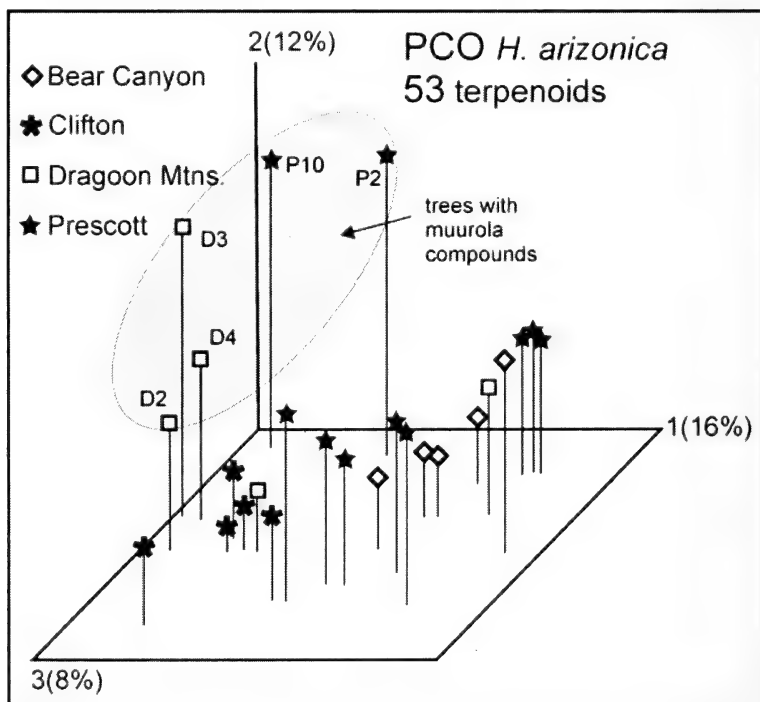
Figure 5. PCO based on 53 terpenoids of *H. arizonica* based on leaf oils. Note the Dragoon Mtns. and Prescott populations are di-morphic for the muurola compounds.

the Dragoon Mtns. had the muurola suite of compounds but the other two trees had absolutely no traces of the muurola compounds. In general, the muurola compounds were found in trace values in two trees from the Bear Canyon and two trees from the Clifton populations. The genes for the muurola pathway seem to be widespread in *H. arizonica*. The muurola compounds are characteristically in large concentrations in *H. glabra*. The presence of the muurola genes in some *H. arizonica* trees could be explained by past hybridization or relictual ancestral lineage sorting between H. arizonica and H. glabra. The lack of the occurrence of other components of *H. glabra* in any *H. arizonica* plants sampled suggests that hybridization is not occurring at present and favors the relictual ancestral lineage sorting hypothesis.

The discovery of *H. arizonica* near Prescott, outside its historical range and in a very xeric habitat compared to the more mesic habitats in southeastern Arizona is difficult to explain if the stand were natural. The grove near Prescott is very small and consists of only 10 trees larger than 1" DBH plus a few seedlings. As cis-muurola-4(14),5-diene is a characteristic component of the muurola suite, it is used to illustrate the diversity in Table 2. We have identified 3 age classes in the grove (approximated by DBH: 20-22", 5.6-11.1", seedlings - 1.6",Table 2). The two trees highest in cis-muurola-4(14),5-diene (and other muurola components) are P2 (18" DBH) and P10 (1"DBH). Recall that P2 and is most similar in its oils to D3 from the Dragoon

Table 2. Analyses of the 10 largest trees from the Prescott population (DBH>1"). % CM45 = % cis-muurola-4(14),5-diene.

Tree #	Rings	Size (DBH)	% CM45
P1	33-40	11.1"	0.3
P2	63-70	20.4"	3.5
P3		21-22"(3 trunks)	0.1
P4	30-44	6.4"	0.2
P5		5.6"	0.05
P6		21.3"	0.0
P7		2.1"	0.05
P8		1.6"	0.05
P9		7.5"	0.0
P10		0.5"	3.8

Mtns. Most of the trees contain small or trace amounts of cis-muurola-4(14),5-diene (Table 2). A spatial analysis of these 10 trees is shown in Fig. 6.
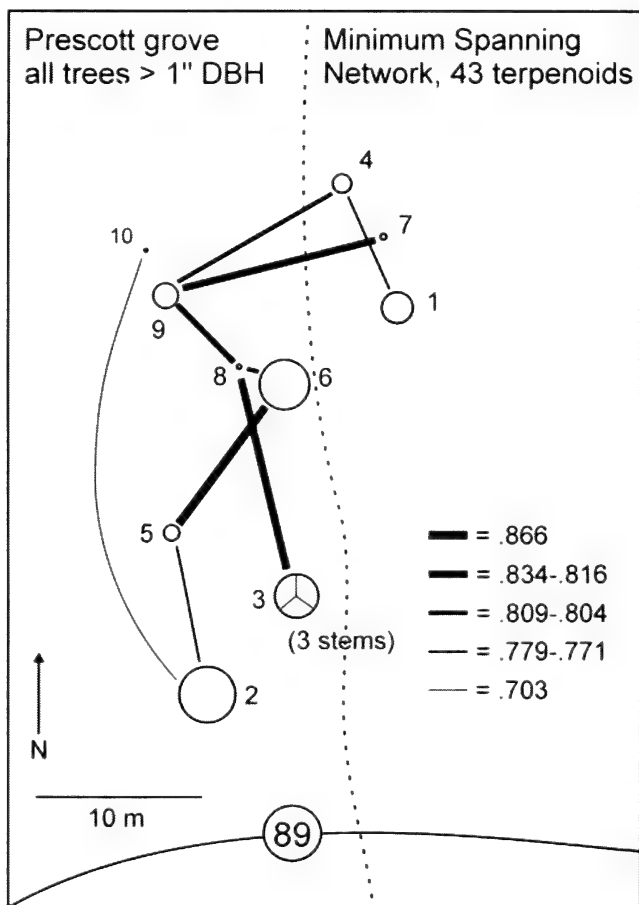

Figure 6. Minimum spanning network (based on 43 terpenoids) for the 10 largest trees in the Prescott grove. The size of the circles is proportional to the DBH (tree 2 = 18" DBH, tree 10 = 1" DBH). Highway 89 is noted by the circled number.

Although trees P2 and P10 share the muurola suite of components, their oils are the least similar of the 10 trees (Fig. 6). Based on field observations in this area, the population appears to have been founded by 3 trees (P2, P3, P6, Fig. 6). Both trees P2 and P3 have dead tops and are declining in health. No additional cypress trees were found in a survey of the ravine above and below this population. There is some litter and topsoil under the trees and new seedlings have been established under the canopy of the large, older trees. No seedlings were seen in the more xeric slopes outside the canopy of the older trees. Growth rings are not very useful due to droughts (no ring that year) and multiple monsoon events (multiple rings/ year). However, coring 3 trees in the population (Table 2) gave a range of ages of 63 - 70 years for the largest tree (#2). If 70 years were true, that would put the origin around 1940. Even allowing for a 50% error (70+35 = 105), that would put the origin at 1905. Historical records record that this section of US Highway 89 was known (and still is) as the White Spar Road. The White Spar Mine (Barite) was located south of the cypress grove in 1880 with claims patented in 1904. In 1927, the White Spar Road became part of US Highway 89. Because *H. arizonica* trees in the Dragoon Mtns. are easily accessible and cones are easily collected, *H. arizonica* could have been brought to the Prescott area in the 20th century.

In light of the above discussion, the Prescott population appears to be an anthropogenic introduction. This conclusion should not be unexpected given that both Arizona cypresses, *H. arizonica* and particularly, *H. glabra*, are very commonly cultivated in Arizona outside their respective native ranges was well as elsewhere throughout the United States and the world. Identified generically in the horticultural community as "Arizona Cypress", both species are used as ornamentals, windbreak trees and sometimes on disturbed sites for erosion control (Sullivan, 1993). While *H. glabra* has been cultivated to a greater degree given its comparative better hardiness and desirability as a Christmas tree (Jacobson, 1996), both species have been cultivated for more than a century; *H. arizonica* since at least 1882 (Dallimore and Jackson, 1966) and *H. glabra* since as early as 1888 (Peattie, 1953). Interestingly, *H. glabra* was in cultivation prior to its description in 1907, which may have contributed to the confusion of the two species in cultivation.

To examine infraspecific variation in *H. glabra*, the terpenoids similarity matrix was factored. The first three eigenroots accounted for 16.6%, 10.9% and 8.8% of the variance among the 26 samples. Ordination revealed that the unusual nature of two of the Mazatzal Mtns. plants and the divergence of the Yarnell population (Fig. 7). The Kirkland, Sedona and East Verde River populations are interspersed in
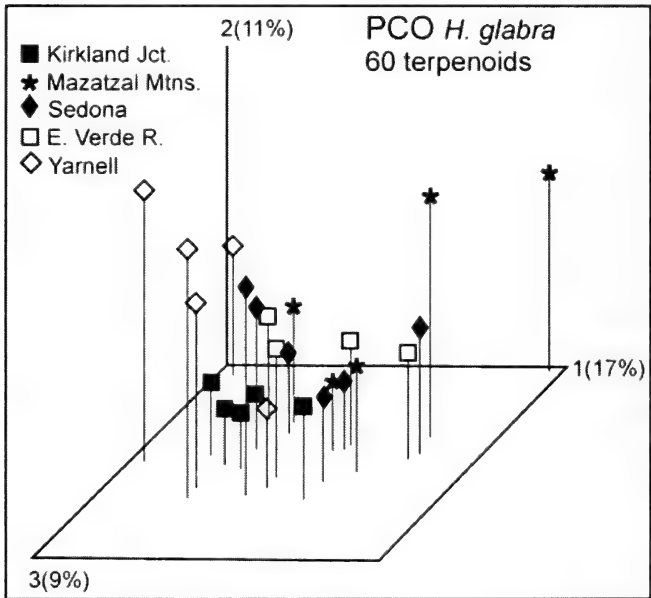

Figure 7. PCO of *H. glabra* individuals based on 60 terpenoids.

the ordination (Fig. 7). It should be noted that one of the Yarnell plants and three of the Mazatzal Mtns. plants are interspersed with typical *H. glabra* (Fig. 7). All of these five populations are relatively near (15-60 mi.), so pollen flow is possible.

CONCLUSIONS

Two chemotypes were found in *H. arizonica*: low muurola trees (typical of the species) and a few high murrola trees. A disjunct, population near Prescott has both chemotypes as also found in the Dragoon Mtns. population. The leaf oil of one of the Prescott 'high

muurol' chemotype individuals (P2) was found to be most similar to a tree from the Dragoon Mtns., indicating that the Prescott genotypes came from southeastern Arizona. The Prescott *H. arizonica* population appears to have been introduced by man with germplasm (seed cones) from the Dragoon Mtns. or an adjacent area. The unusual amount of variation found in the Prescott (*H. arizonica*) and Yarnell (*H. glabra*) populations is puzzling and deserves additional study.

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for *H. arizonica* (ariz), *H. glabra* (glab), and putative introgressant from Prescott (12079,P2) and unusual oils from plants from the Mazatzal Mtns. (11680, M1) and Yarnell (12085, Y3). Compounds with an asterisk (*) were used in numerical analysis. Compounds in bold show large differences between the taxa. F (from ANOVA) * = 0.05; ** = 0.01; *** = 0.001.

RT	compound	ariz	glab	F	D3	P2	M1	Y3
846	(E)-2-hexenal	0.4	0.3	ns	t	-	0.5	0.2
921	tricyclene	0.1	0.1	ns	t	t	t	t
924	**α-thujene***	**0.9**	**0.6**	**36*****	**1.8**	**1.3**	**1.1**	**0.1**
932	**α-pinene***	**3.9**	**12.6**	**14*****	**3.5**	**7.7**	**10.6**	**8.7**
945	α-fenchene	-	0.1	ns	-	-	0.1	0.1
946	camphene	t	0.2	ns	t	t	0.1	0.1
953	thuja-2,4-diene	-	t	nt	-	-	-	0.1
969	sabinene*	4.3	3.4	ns	8.2	6.1	9.6	1.1
974	β-pinene*	0.2	0.4	nt	0.2	0.3	0.4	0.4
988	myrcene*	1.7	1.7	6.9*	2.3	2.9	3.9	0.8
994	2-octanol	t	t	nt	-	-	-	-
1002	α-phellandrene	0.1	0.2	ns	0.2	0.2	0.2	t
1008	δ-3-carene*	0.3	1.8	ns	0.1	0.1	1.1	0.9
1014	**α-terpinene***	**1.4**	**0.7**	**168*****	**1.9**	**1.4**	**1.3**	**0.2**
1020	p-cymene*	1.1	0.7	28***	2.0	1.5	1.0	0.4
1024	limonene*	4.2	4.0	ns	5.9	6.1	15.3	0.7

RT	compound	ariz	glab	F	D3	P2	M1	Y3
1025	β-phellandrene*	4.2	4.0	ns	5.9	6.1	15.3	0.7
1026	1,8-cineole	t	-	nt	-	-	-	-
1038	2-heptyl acetate	t	-	nt	-	-	-	-
1054	**γ-terpinene***	**1.8**	**1.1**	**119*** **	**2.5**	**2.3**	**1.8**	**0.3**
1065	cis-sabinene hydrate*	0.6	0.2	32***	1.0	0.7	0.6	0.1
1086	**terpinolene***	**1.7**	**1.0**	**62*** **	**1.7**	**1.6**	**1.7**	**0.5**
1087	2-nonanone	t	-	nt	t	-	-	-
1098	trans-sabinene hydrate*	0.6	0.1	28***	0.8	0.5	0.3	0.1
1099	linalool*	0.3	0.2	nt	0.3	0.4	0.2	t
1100	n-nonanal	t	-	nt	t	-	-	0.2
1112	trans-thujone	t	-	nt	-	-	-	-
1118	**cis-p-menth-2-en-1-ol***	**0.7**	**0.2**	**84*** **	**0.9**	**0.5**	**0.3**	**0.1**
1122	α-campholenal	-	0.1	nt	-	-	t	0.1
1136	trans-p-menth-2-en-1-ol*	0.5	0.2	28***	0.6	0.4	0.3	0.5
1141	camphor*	0.6	1.3	ns	0.2	0.3	0.3	0.3
1145	camphene hydrate	0.3	-	nt	0.4	0.3	t	t
1148	citronellal	-	t	nt	-	-	0.2	t
1160	pinocarvone	-	-	nt	-	-	-	0.2
1167	**umbellulone***	**19.0**	**8.8**	**88*** **	**22.7**	**15.7**	**3.7**	**3.9**
1174	**terpinen-4-ol***	**5.9**	**2.7**	**76*** **	**8.2**	**7.8**	**3.8**	**0.9**

RT	compound	ariz	glab	F	D3	P2	M1	Y3
1179	p-cymen-8-ol*	1.3	0.5	6.4*	1.8	0.8	0.4	0.2
1186	α-terpineol*	0.7	0.5	ns	0.5	0.4	0.2	0.2
1195	cis-piperitol	0.2	-	nt	0.3	-	0.1	0.1
1195	myrtenal	-	0.1	nt	-	-	0.1	-
1198	shisofuran	0.2	-	nt	0.2	0.2	-	-
1205	trans-piperitol	0.3	0.3	nt	0.3	0.2	-	-
1206	verbenone	-	0.2	nt	-	-	-	-
1215	trans-carveol	t	-	nt	-	-	-	-
1223	citronellol*	0.1	2.4	18**	t	0.1	3.6	0.1
1232	thymol, methyl ether	0.1	t	nt	-	0.5	0.2	0.3
1239	carvone	t	t	nt	t	-	0.1	0.3
1241	carvacrol, methyl ether	t	-	nt	-	0.1	-	-
1249	piperitone	0.2	t	nt	t	0.2	0.1	t
1254	linalool acetate	0.1	-	nt	-	0.1	-	-
1287	bornyl acetate	0.1	-	nt	0.6	0.1	-	-
1289	thymol	0.2	t	nt	t	0.1	t	0.2
1293	**2-ethyl-isomenthone***	-	**0.2**	**49*****	-	-	**0.1**	**0.9**
1299	terpinen-4-yl acetate*	0.9	0.5	19**	1.3	1.3	0.5	0.5
1315	(2E,4E)-decadienal	t	-	nt	-	-	-	-
1319	(2E,4E)-decadienol	t	-	nt	-	-	-	-

RT	compound	ariz	glab	F	D3	P2	M1	Y3
1339	trans-carvyl acetate	-	t	nt	t	-	0.1	-
1346	**α-terpinyl acetate***	**2.6**	**0.6**	**139*****	**3.0**	**4.2**	**0.7**	**0.2**
1373	α-copaene	t	-	nt	-	-	-	-
1410	α-cedrene*	-	0.5	nt	-	-	-	0.3
1419	β-cedrene*	-	0.3	nt	-	-	-	0.5
1444	bakerol	-	-	nt	-	-	-	0.2
1448	**cis-muurola-3,5-diene***	**0.2**	**4.3**	**79*****	**1.7**	**1.5**	**2.0**	**3.2**
1452	α-humulene	-	t	nt	-	-	-	0.2
1465	**cis-muurola-4(14),5-diene***	**0.5**	**11.8**	**80*****	**4.0**	**3.5**	**5.0**	**8.0**
1479	ar-curcumene	-	-	nt	-	-	t	0.2
1482	citronellol isobutyrate	-	0.2	nt	-	-	0.1	-
1499	β-macrocarpene	-	-	nt	-	-	-	0.2
1500	α-muurolene	0.1	-	nt	-	-	-	-
1501	**epi-zonarene***	**t**	**3.9**	**80*****	**1.0**	**0.9**	**1.4**	**2.1**
1504	cuparene	-	-	nt	-	-	-	0.2
1512	**α-alaskene***	**-**	**0.2**	**nt**	**-**	**-**	**t**	**0.3**
1513	**γ-cadinene**	**0.2**	**-**	**nt**	**-**	**-**	**-**	**-**
1518	endo-1-bourbonanol	t	-	nt	-	-	-	t
1521	**trans-calamenene***	**t**	**2.7**	**84*****	**0.2**	**0.1**	**0.6**	**3.9**
1522	**δ-cadinene***	**0.8**	**t**	**12****	**0.2**	**0.1**	**t**	**-**

RT	compound	ariz	glab	F	D3	P2	M1	Y3
1533	**10-epi-cubebol***	-	**0.4**	**43***	-	-	**t**	**0.2**
1536	**italicene ether***	-	**0.9**	**25****	-	**0.1**	**0.2**	**1.5**
1537	α-cadinene	t	-	nt	-	-	-	-
1544	α-calacorene	-	0.2	nt	-	-	-	0.2
1548	elemol	0.2	-	nt	0.1	-	-	-
1550	**cis-muurola-5-en-4-β-ol***	**0.1**	**1.6**	**44***	**0.5**	**0.5**	**1.0**	**0.9**
1559	**cis-muurola-5-en-4-α-ol***	**0.2**	**1.9**	**35***	**0.7**	**0.7**	**1.2**	**1.2**
1561	**germacrene D-4-ol**	**0.9**	-	**19***	**0.2**	**0.1**	-	-
1564	β-calacorene	-	0.1	nt	-	-	-	0.1
1582	caryophyllene oxide	-	t	nt	-	-	t	-
1600	**cedrol***	-	**1.2**	**41***	-	**t**	**t**	**3.2**
1607	β-oplopenone	0.2	-	nt	t	0.1	-	-
1608	humulene epoxide II	t	0.1	nt	-	-	t	-
1618	**1,10-di-epi-cubenol***	**0.1**	**0.4**	**81***	**t**	**0.1**	**0.1**	**0.4**
1627	1-epi-cubenol	t	-	nt	t	-	-	-
1627	**3-oxobutyl-isomenthone***	-	**0.6**	**nt**	-	-	**t**	**2.3**
1632	**α-acorenol***	-	**3.2**	**61***	-	-	**0.2**	**3.8**
1636	**β-acorenol***	-	**0.6**	**59***	-	-	**t**	**0.8**
1638	epi-α-cadinol*	0.6	0.3	nt	0.1	0.1	0.1	0.3
1638	epi-α-muurolol*	0.6	0.3	nt	0.2	0.1	0.1	0.3

RT	compound	ariz	glab	F	D3	P2	M1	Y3
1644	α-muurolol	0.2	-	nt	-	-	-	-
1645	cubenol	-	t	nt	-	-	-	-
1652	α-cadinol*	1.6	0.9	ns	0.3	0.4	0.2	0.8
1661	**terpenoid 43,139,155,238***	-	**0.6**	**55*****	-	-	**0.1**	**1.6**
1675	**cadalene***	-	**0.5**	**24*****	-	-	**0.1**	**1.2**
1685	α-bisabolol	t	-	nt	-	-	-	-
1688	**cis-14-nor-muurol-5-en-4-one***	**0.1**	**4.0**	**46*****	**0.2**	**0.1**	**0.7**	**9.5**
1740	(E)-isoamyl cinnamate	0.1	-	nt	-	t	-	-
1748	(Z)-isoamyl cinnamate	0.2	-	nt	-	0.1	-	-
1887	**oplopanonyl acetate**	**1.0**	**0.1**	**25****	**0.1**	**0.2**	-	-
1905	isopimara-9(11),15-diene	0.4	-	nt	0.3	0.4	-	0.3
1933	**isohibaene***	**0.9**	**t**	**68*****	**0.2**	**0.6**	-	**0.3**
1941	sandaracopimara-8(14),15-diene	0.3	-	nt	t	0.2	-	-
1960	iso-sandaracopimara-8(14),15-diene	1.0	t	nt	0.1	-	0.2	0.6
1966	**isophyllocladene***	**3.7**	**0.4**	**36*****	**1.8**	**4.2**	**t**	**1.0**
1978	manoyl oxide*	2.0	0.8	ns	1.0	1.8	0.7	3.7
1987	**13-epi-manoyl oxide***	**0.5**	**t**	**5.9***	**0.2**	**0.4**	**0.2**	**1.4**

RT	compound	ariz	glab	F	D3	P2	M1	Y3
2034	**kaur-16-ene***	**0.4**	-	**nt**	**0.1**	**0.3**	**t**	-
2055	abietatriene*	0.2	0.2	nt	-	0.1	0.2	0.3
2087	abietadiene*	0.4	0.9	4.6*	-	t	0.2	0.5
2090	diterpene, <u>55</u>,41,272,290*	0.4	-	nt	0.1	0.3	-	0.4
2105	isoabienol	0.6	0.1	nt	0.2	-	-	-
2132	**nezukol***	**15.2**	**0.6**	**42***	**7.1**	**11.0**	**1.6**	**14.8**
2209	phyllocladanol*	1.3	0.5	ns	t	t	-	0.8
2282	sempervirol	0.3	0.1	nt	t	0.5	0.2	0.4
2314	trans-totarol	0.2	0.1	nt	t	0.3	0.2	0.4
2331	trans-ferruginol	0.1	t	nt	t	0.2	t	0.3

AI = Arithmetic Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

FOUR NEW SPECIES OF *AGERATINA* (ASTERACEAE: EUPATORIEAE) FROM OAXACA, MEXICO

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ABSTRACT

Routine identification of Mexican Asteraceae has revealed the following novelties in the genus *Ageratina*:   **A. hasegawana** B.L. Turner **sp. nov.**. **A. huahuapana** B.L. Turner, **sp. nov. A. megacephala** B.L. Turner, **sp. nov**. and **A. ozolotepecana** B.L. Turner, **sp. nov.** All of the taxa are from the state of Oaxaca, and all belong to the subgenus *Neogreenella*. Phototypes are provided, along with maps showing their distribution. *Phytologia 92(3): 388-399 (December 1, 2010).*
KEY WORDS: Asteraceae, *Ageratina*, Mexico, Oaxaca

Ageratina is perhaps the most speciose genus of the Asteraceae in Mexico, at last count (Turner 2008) possessing some 149 species; the present contribution brings the total to 153.

AGERATINA HASEGAWANA B.L. Turner, sp. nov., Fig. 1

Ageratinae deltoideae (Jacq.) King & H. Rob. similis sed differt foliis plerumque minoribus (5-8 cm in latitudine vs 8-14 cm) subter dense villosis secus venas majores (vs. non villosis), capitulescentiis minus congestis, capitulis minoribus flosculis paucioribus (ca. 25 vs. 30 vel plus), et bracteis involucralibus 12-14 (vs 21 vel plus).

Resembling *Ageratina deltoidea* but the leaves mostly smaller (5-8 cm wide vs 8-14 cm), densely villous beneath along the major veins (vs not so), capitulescence less congested, the heads smaller with fewer florets (ca 25 vs 30 or more), and the involucral bracts 12-14 (vs 21 or more).

Perennial herbs to 1 m (?) high. **Mid-stems** minutely glandular-pubescent. **Leaves** 6-10 cm long, 5-8 cm wide; petioles 2-3 cm long; blades triangular, equilateral, sparsely pubescent above, villous below with matted hairs along the larger veins, the margins crenulate. **Capitulescence** an open cymose panicle, the ultimate, minutely glandular, peduncles 1-2 cm long. **Heads** more or less turbinate, ca 12 mm high, 2-5 mm across, containing 23-25 florets. **Receptacle** glabrous, ca 1.5 mm across. **Involucral bracts** 12-15, narrowly lanceolate, pubescent like the pedicels. **Corollas** white, ca 8 mm long, glabrous, except for occasional long trichomes upon the lobes. **Achenes** black, sparsely hispid, ca 4 mm long; pappus of ca 30 ciliate bristles, 5-6 mm long.

TYPE: **MEXICO. OAXACA:** "155.3 km S of 135/150 junction on Hwy 135 Cuota." 2360 m, 17 36 57 N, 97 19 33 W, 26 Nov 2003, *Yahara T.,Ohnishi W., Hasegawa M., Nakazawa M, & Hiramatsu M. 2783* (Holotype: TEX).

In my treatment of *Ageratina* for Mexico (Turner 1997), *A. hasegawana* will key directly to *A. deltoidea*, to which it is surely closely related; it differs from the latter in numerous characters, as noted in the above diagnosis. It is also well-removed from the distribution of *A. deltoidea* proper, as shown in Fig 5.

The novelty is named for T. Hasegawa of Japan, who participated in its discovery; I have named two other species of *Ageratina* for yet other Japanese workers, *A. soejimana* and *A. yaharana* (Turner 2008), thus honoring the Asian team that has collected a plethora of comps in Mexico over the past decade, mostly in the genus *Stevia*.

AGERATINA HUAHUAPANA B.L. Turner, **sp. nov., Fig. 2**
   Ageratinae viburnoides (DC.) King & H. Rob. similis sed differt foliis subter valde pubescentibus (vs. celeriter glabratis) supra epunctatis (vs. valde punctatis) petiolis majoribus (2-3[4] cm in longitudine vs 0.5-1.5 cm).

Resembling *Ageratina viburnoides* but the leaves markedly pubescent below (vs soon glabrate), epunctate above (vs markedly punctate), with smaller petioles (0.5-1.5 cm long vs 2-3[4] cm).

Perennial suffruticose herbs or subshrubs, 0.5-1.5 m high. **Mid-stems** densely appressed-pubescent. **Leaves** thickened, mostly 5-9 cm long, 4-5 cm wide; petioles 2-3 cm long; blades broadly ovate to deltoid, penninervate, rarely auriculate at base, minutely and densely glandular-pubescent below, markedly glandular-punctate above, the margins entire, or nearly so. **Heads** arranged in terminal congested, cymose-panicles, 4-8 cm high and about as wide. **Involucres** hemispheric, 4-5 mm high, densely pubescent; bracts sub-equal, 10-12 in 2-3 series, the apices often purplish. **Receptacles** plane, ca 1 mm across, glabrous. **Florets** 10-12 per head, the corollas pinkish-white, glabrous. **Achenes** (immature) ca 1.5 mm long; pappus of ca 20 bristles, ca 3 mm long.

TYPE: **MEXICO. OAXACA: Distrito Huahuapan, Mpio. Santo Domongo Tonola,** Paraje "La Taberna en la Canada." Bosque de encino. ca 1962 m, 17 38 14.3 N, 97 56 42.2 W, 18 Oct 2008, *Luis Angel Hernandez Perez 548* [con A. Torres] (Holotype: TEX).

Additional Collection: **same Mpio. as above:** Paraje "La Canada del Rocio." ca 1870 m, 17 43 29.6 N, 97 55 52.4 W, 24 Oct 2008, *Perez 637* (TEX).

In my treatment of *Ageratina* in the Comps of Mexico (Turner 1997), *A. huahuapana* will key to, or near, *A. viburnoides* a thick-leaved, more oval-leafed, penninervate. species of northeastern Mexico. The two taxa are easily differentiated, as noted in the above diagnosis.

·AGERATINA MEGACEPHALA B.L. Turner, **sp. nov., Fig. 1**

Ageratinae aegirophyllae (B.L. Rob.) King & H. Rob. similes sed differt statura breviori (usque ad 0.4 m in altudine vs 1-3 m), capitulis multo majoribus, bracteis involucralibus longioribus, et flosculis numerosioribus (80+ vs 10-30).

Resembling *A. agerophylla* but an herb to 0.4 m high (vs 1-3 m) having much larger heads and more numerous involucral bracts and florets per head.

Perennial herbs to 40 cm high. **Stems** densely glandular-pubescent, the vestiture ca 0.5 mm high. **Leaves** opposite, 15-20 cm long, 4-6 cm wide; petioles 3-5 cm long, more or less clasping at very base; blades broadly lanceolate to ovate-elliptic, 3-nervate ca 1-2 cm from the base; grading into the petioles, glandular-pubescent below, mostly along the veins. **Capitulescence** a terminal corymbose panicle ca 8 cm high, and as wide, the ultimate peduncles 1-3 cm long, pubescent like the stems. **Heads** ca 15 mm high, 20 mm across, the involucral bracts ca 10 mm long, glandular-pubescent, linear-lanceolate, numerous, in 2-3 equal series; receptacle plane, sparsely pubescent, 5-6 mm across. **Disk florets** numerous; corollas white, glabrous, or nearly so, ca 8 mm long; tube ca 5 mm long; throat 2-3 mm long, grading into the tube, the lobes ca 0.4 mm long, glabrous or nearly so. **Anthers** yellow, ca 2.5 mm long, the appendages longer than wide. **Style branches** linear, exserted for 5-7 mm. **Achenes** black, 5-ribbed, pubescent with upwardly-projected, stiff hairs; pappus of ca 20 barbellate bristles, 6-7 mm long.

 TYPE: **MEXICO. OAXACA: Distrito Putla. Mpio. Santa Cruz Itundujia,** "Rio Tigre, a 5 km al NE en LR de la Agencia de Zaragoza." Selva mediana subcaducifolia. ca 1021 m, 16 41 10.5 N, 97 45 10 W, 16 Apr 2008, *Kenia V. Gutierrez 2617* [con Sr. Elias].

This is a very distinct species of Ageratina. In my treatment of *Ageratina* for Mexico (Turner 1997), this novelty will key directly to *A. aegirophylla*, which it scarcely resembles, differing especially in its much larger heads (whence the name), and larger, more lanceolate, leaves.

AGERATINA OZOLOTEPECANA B.L. Turner, **sp. nov., Fig. 3**

Ageratinae havanensi (H.B.K.) King & H. Rob. similis sed differt capitulis majoribus (10-12 mm in altitudine vs 5-6 mm), floribus numerosioribus (ca 25 vs 5-20) et bracteis involucralibus longioribus numerosioribus glandulosi-pubescentibus.

Resembling *Ageratina havanensis* (H.B.K.) King & H. Rob. but having larger heads (10-12 mm high vs 5-6 mm), more numerous florets (ca 30 vs 5-20), and longer, more numerous, glandular-pubescent involucral bracts.

Shrub 2 m high. **Stems** minutely puberulent to glabrate. **Leaves** mostly 3-4 cm long, 1.5-2.5 cm wide; petioles 5-10 mm long; blades ovate, glabrous, without glandular-punctations beneath, the margins serrate. **Capitulescence** cymose paniculate, terminal, ca 5 cm high, 3 cm across, the ultimate peduncles 6-10 mm long. **Heads** 10-12 mm high; involucral bracts 8-9 mm long, linear-lanceolate, purplish, minutely glandular-pubescent, **Receptacles** plane, glabrous, ca 2 mm across. **Florets** ca 25 per head; corollas white, glabrous, ca 6 mm long, the lobes glabrous. **Achenes** black, ca 3 mm long, sparsely pilose; pappus of ca 30, rosy-white, persistent, bristles ca 6 mm long.

TYPE: **MEXICO. OAXACA: Distrito Miahuatlan. Mpio. San Juan Ozolotepec,** "Bosque de pino., sobre cerro." ca 3408 m, 16 11 37 N, 96 16 3.4 W, 3 Dec 2009, *Silvia H. Salas M 6618* [con A. Sanchez, F. Maldonado & J. Lucas] (Holotype: TEX).

In my treatment of *Ageratina* for Mexico (Turner 1997) *A. ozolotepecana* will key directly to *A. havanensis*, to which it is surely closely related. It differs from the latter in numerous characters, as noted in the above diagnosis.

The distribution of *A. havanensis* in Mexico is shown in Fig.6; it occurs largely along the more eastern sierras of Mexico, mostly from 700-1400 m. *A. ozolotepecana* reportedly occurs in pine forests above 3000 m, presumably in cloud forests, to judge from the lichens that enwrap some of the stems on the type specimen.

Named, in part, for the Mpio. San Juan Ozolotepec, whence the type.

ACKNOWLEDGEMENTS

I am grateful to my colleague Guy Nesom for the Latin diagnosis and reviewing the manuscript.

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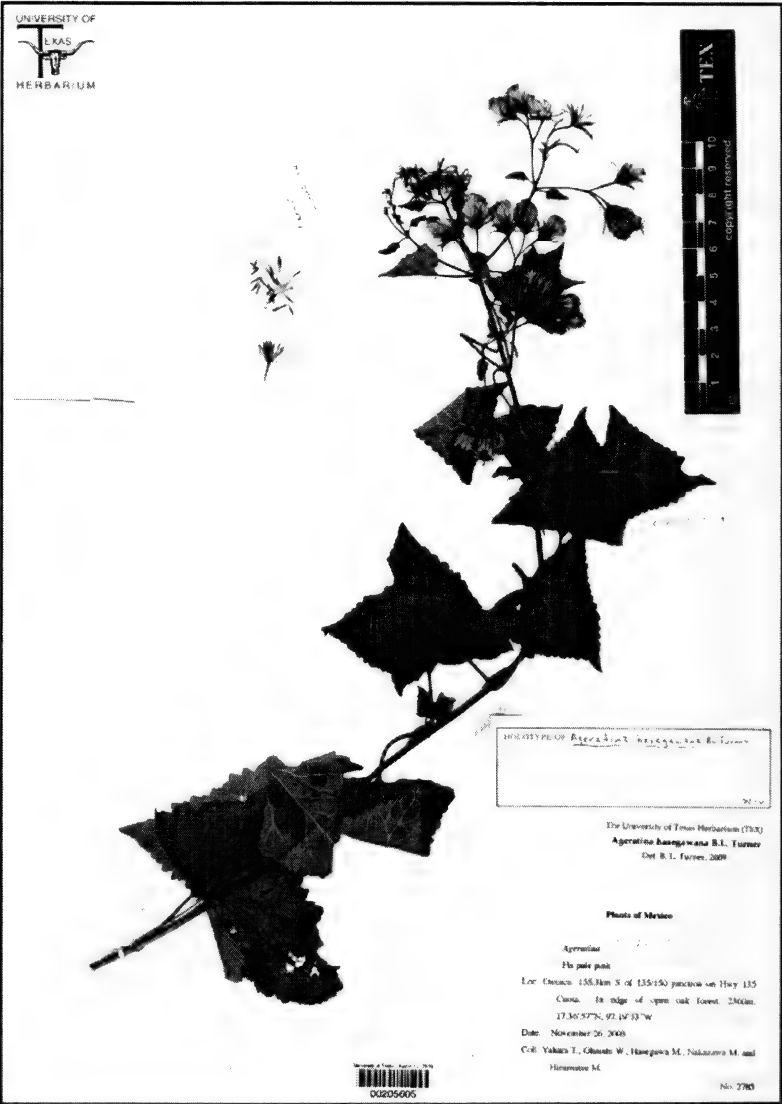
Fig. 1. *Ageratina hasegawana* (Holotype).
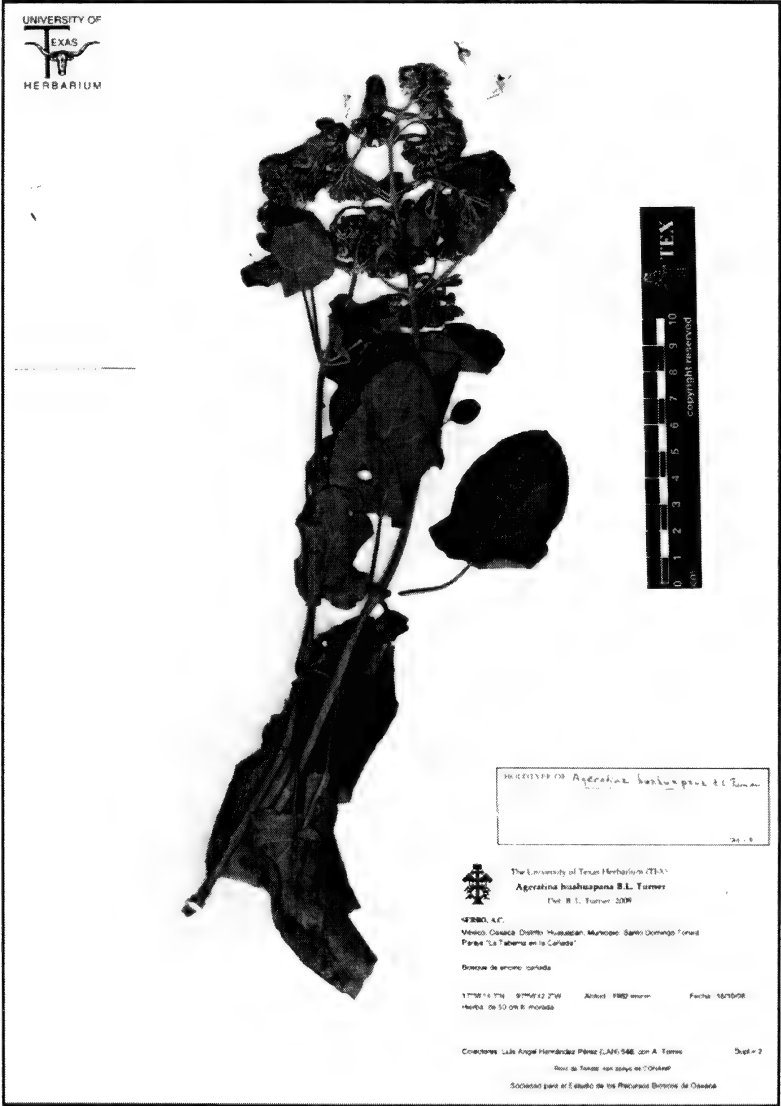
Fig. 2. *Ageratina huahuapana* (Holotype).
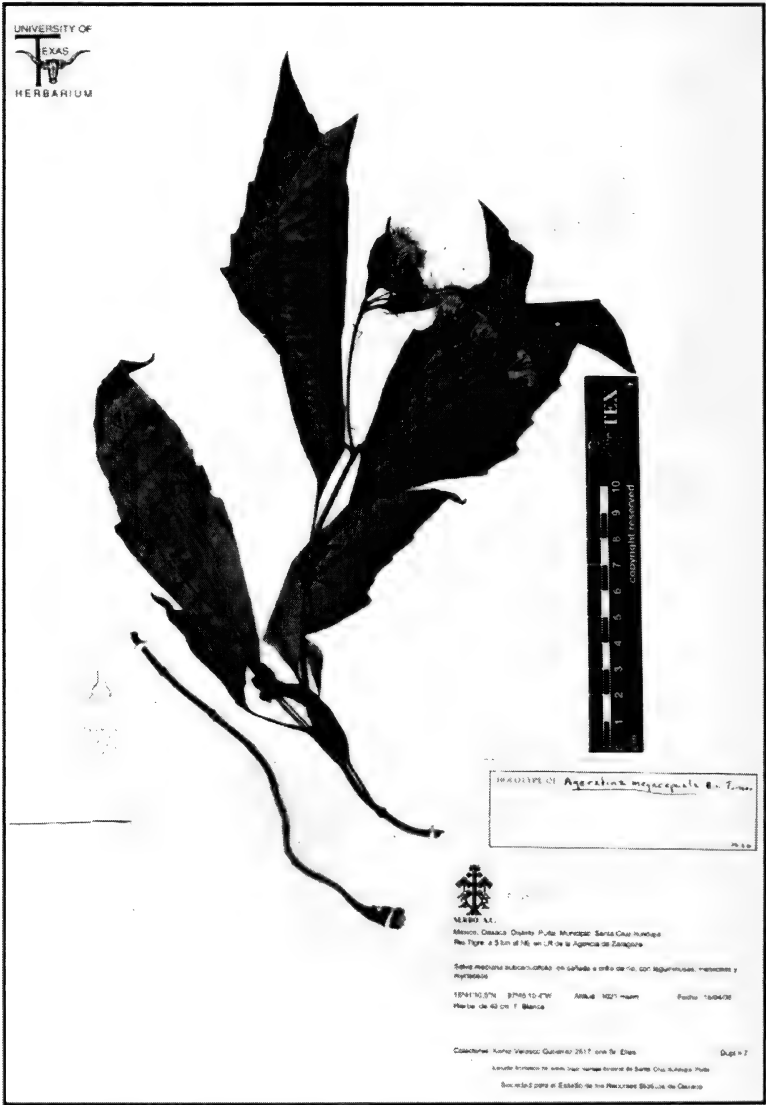
Fig. 3. *Ageratina megacephala* (Holotype)
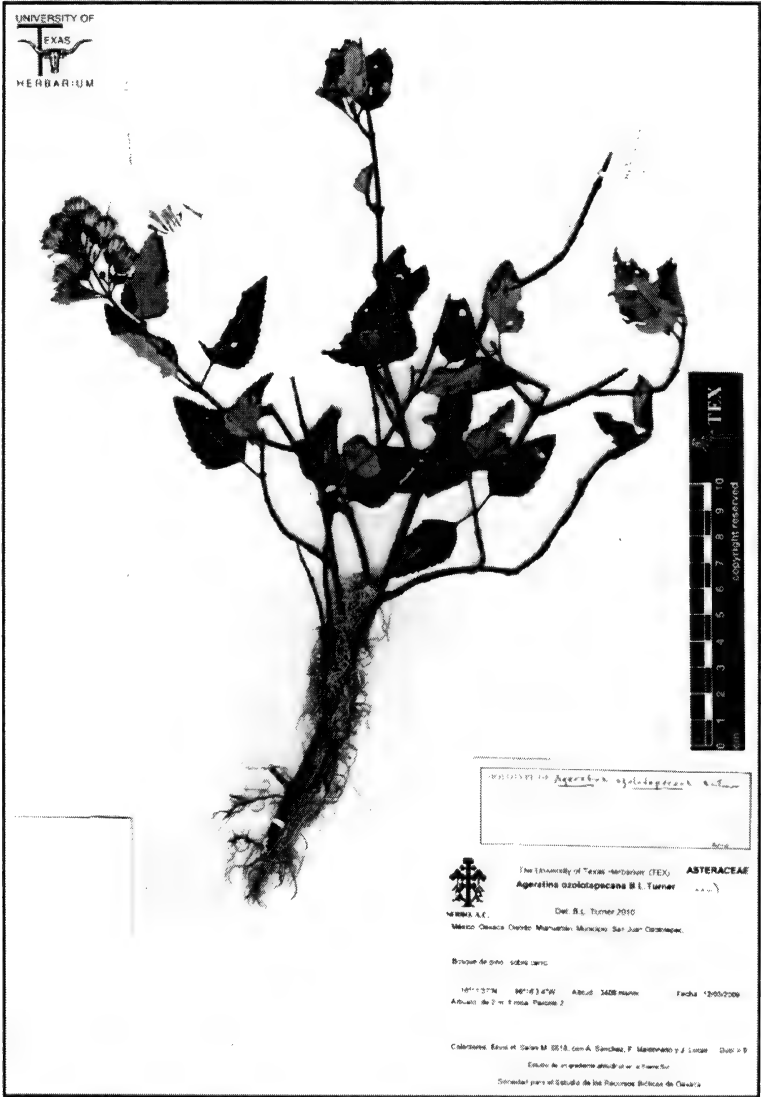
Fig. 4. *Ageratina ozolotepecana* (Holotype).
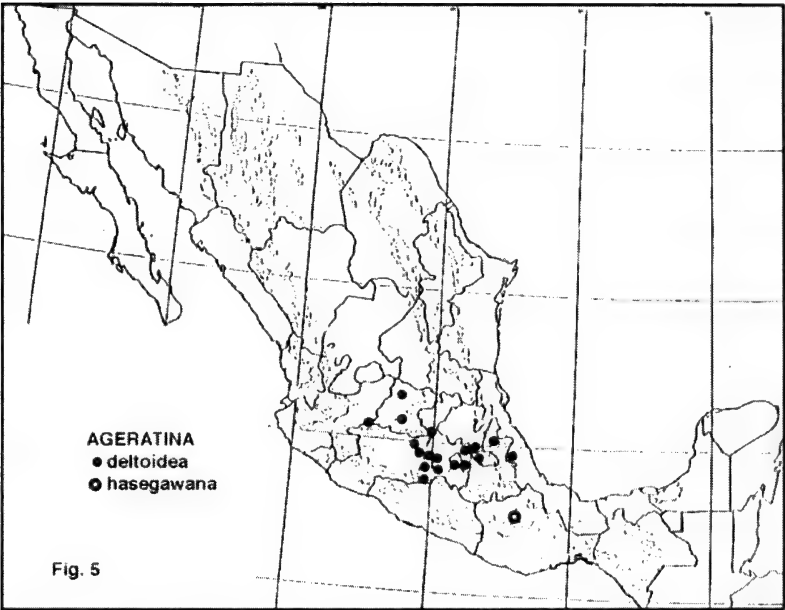
Fig. 5. Distribution of *Ageratina hasegawana* and *A. deltoidea*.
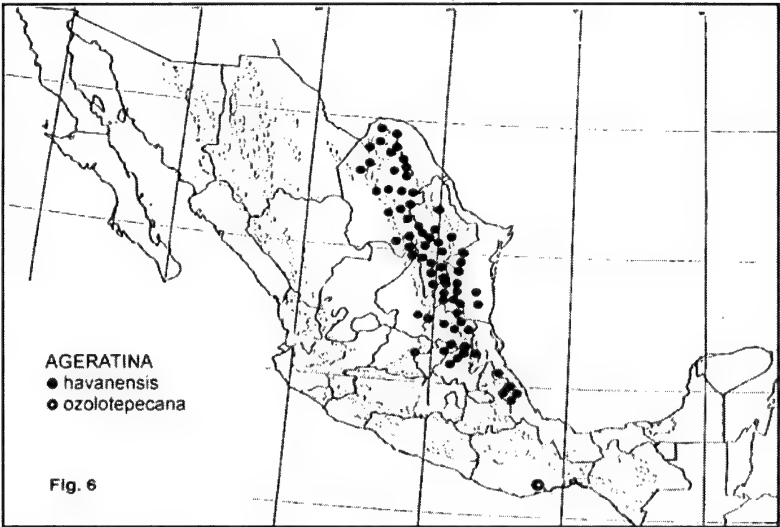
Fig. 6. Distribution of *Ageratina havanensis* (in Mexico) and *A. ozolotepecana*.

QUERCUS HINCKLEYI × *Q. VASEYANA,*
A PUTATIVE HYBRID FROM PRESIDIO COUNTY, TEXAS

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ABSTRACT

Quercus hinckleyi C. H. Mull. occupies a small geographic range in Presidio County, Texas, where it is sympatric with *Q. pungens* Liebm. and *Q. vaseyana* Buckl. A specimen of *Quercus*, recently discovered growing near the perimeter of a subpopulation of *Q. hinckleyi*, exhibits morphological and morphometric features of both *Q. hinckleyi* and *Q. vaseyana*. This paper reviews the morphology – with emphasis on leaf morphology – of the latter two species and reports new morphometric data on their leaves. Corresponding data collected from the unusual *Quercus* specimen are then compared and contrasted with those data. Based on this comparative analysis, the peculiar *Quercus* individual appears to constitute the first reported case of natural hybridization between *Q. hinckleyi* and *Q. vaseyana*. *Phytologia 92(3): 400-406 (December 1, 2010).*

KEY WORDS: *Quercus hinckleyi, Q. vaseyana, Q. hinckleyi* × *Q. vaseyana*, hybrid, leaf morphology.

   Quercus hinckleyi C. H. Mull. (Hinckley oak) is a rare, diminutive (≤1 m high) oak known only from limestone and sandstone substrates at elevations of 1070-1370 m in southern Presidio County and adjacent Brewster County, Texas, where it is sympatric with two other oaks, *Q. pungens* Liebm. and *Q. vaseyana* Buckl., which show more extensive geographic distributions (Powell, 1998; Turner et al.,

2003; Poole et al., 2007). Muller (1951, p. 71) treated these two species of oaks as only varietally distinct, which was "demanded by the large number of intermediates occurring in Brewster Co." We follow Nixon (1997), however, in his treatment of *Q. pungens* and *Q. vaseyana* as distinct species.

Quercus hinckleyi was federally listed as threatened in 1988, principally because of its restricted geographic distribution, small population size, and low reproductive rate (U.S. Fish and Wildlife Service, 1988), and "very little" data have been generated on the species in recent years (U.S. Fish and Wildlife Service, 2009). Although hybridization among oak species is common (Powell, 1998), no clear instance of hybridization of *Q. hinckleyi* with any other species has been reported to date. Here we describe a specimen that appears to be an F_1 hybrid between *Q. hinckleyi* and *Q. vaseyana*.

MATERIALS AND METHODS

While collecting leaf samples from a population of *Q. hinckleyi* near Shafter, Texas, we discovered a single mature individual oak bearing two types of leaves on any given branch: small distal leaves grossly resembling the leaves of *Q. hinckleyi* and large proximal leaves with some gross resemblance to the leaves of *Q. vaseyana*. This apparent hybrid plant was growing approximately five m downslope from a motte of typical *Q. hinckleyi* on a ridge composed of limestone and sandstone strata. This plant was approximately 4 m in height (Fig. 1a), in marked contrast to the nearby *Q. hinckleyi*, none of which exceeded 1 m in height (Fig. 1b). We were unable to locate a specimen of *Q. vaseyana* within a 50-m radius of the putative hybrid, but the taxon is known to occur in the Shafter area. Gross and microscopic observations of leaf morphology of the two parental species and the putative hybrid, as well as morphometric data on the leaves, were based on herbarium specimens at SRSC, including three from the vicinity of Shafter: *Q. hinckleyi* (*S. Weyerts 104*); *Q. vaseyana* (*A.M. Powell & S. Powell 3733*); and *Q. hinckleyi* × *Q. vaseyana* (*M.K. Terry 950*).

RESULTS AND DISCUSSION

As with most oaks, the most informative findings that distinguished *Q. hinckleyi* and *Q. vaseyana* consisted of differences in leaf morphology. Fig. 2a shows the gross leaf morphology of a typical *Q. hinckleyi* specimen, *S. Weyerts 104*. Fig. 2c shows that of a typical *Q. vaseyana* specimen, *A.M. Powell & S. Powell 3733*. Table 1 shows comparative leaf data on *Q. hinckleyi* and *Q. vaseyana*.

Fig. 2b shows two conspicuously different leaf types on the same branch from a herbarium specimen of the putative hybrid, *M.K. Terry 950*. At the apices of the branches, the nodes bear small (0.5−1.5 cm long) leaves that look superficially similar to *Q. hinckleyi* leaves, while the proximal nodes bear large (3−4 cm long) leaves that look somewhat similar in size and lobing to leaves of *Q. vaseyana*.

The marked morphological differences between *Q. hinckleyi* and *Q. vaseyana* extend to characters of microscopic leaf morphology. Fig. 3a shows the absence of stellate hairs on a typical abaxial leaf surface of *Q. hinckleyi* (sparse stellate hairs can occur in this species, but uncommonly). Fig. 3c shows abundant (almost confluent on the veins) stellate hairs on the abaxial leaf surface of *Q. vaseyana*. Fig. 3b shows an intermediate character state, viz., sparse occurrence of stellate hairs on the abaxial leaf surface, which is typical of the putative hybrid.

The conspicuous qualitative and quantitative differences between *Q. hinckleyi* and *Q. vaseyana* in their foliar character states (Table 1) could conceivably render it difficult to recognize a hybrid between the two with any certainty. In this instance, however, we have clear intermediate character states (e.g., the intermediate density of stellate hairs in the putative hybrid), plus the unusual situation in which the putative hybrid bears two types of leaves on the same branch, recognizable as representing both of the disparate parental leaf types. Given the unique morphology of *Q. hinckleyi* and the limited number of other oak species in the Shafter area, there is little room for doubt as to the identity of the putative parents of the plant concerned. Microsatellite data, currently under study, should provide further information as to the parentage of the plant in question.

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Table 1. Comparisons of taxonomically valuable foliar characters exhibited by *Q. hinckleyi*, *Q. vaseyana*, and the putative hybrid *Q. hinckleyi* × *Q. vaseyana*

Leaf Trait	*Q. hinckleyi* (*n* = 35)	*Q. vaseyana* (*n* = 124)	Hybrid (*n* = 1)
Length (mm) of blade	12.7 ± 2.8 (7−)8−15(−19)	37.0 ± 9.8 (≤ 60)	27.9 ± 33.7 (≤ 40)
Width (mm) of blade	9.1 ± 2.0 6−12 (−15)	15.6 ± 4.8 (≤ 40)	19.1 ± 29.9 (≤ 31)
Shape	subrotund to ovate	oblong-elliptic to lanceolate	oblong-ovate
Apical teeth	spinose	mucronate	mucronate-spinose
Apex	acute or obtuse	mostly acute rarely obtuse	acute
Base	cordate or auriculate	cuneate to rounded, rarely subcordate	rounded, slightly cordate
Margins	strongly crisped, slightly revolute	flat or somewhat crispate	flat
Lobing	2−4	4−8 prominent lobes	4−6 lobes to almost no lobing
Toothing	spinose	mucronate, variable	cuspidate or mucronate
Veins	raised on both surfaces, more prominent and red basally beneath	raised on both surfaces, more prominent beneath	almost flat on upper surface, markedly raised and red basally beneath
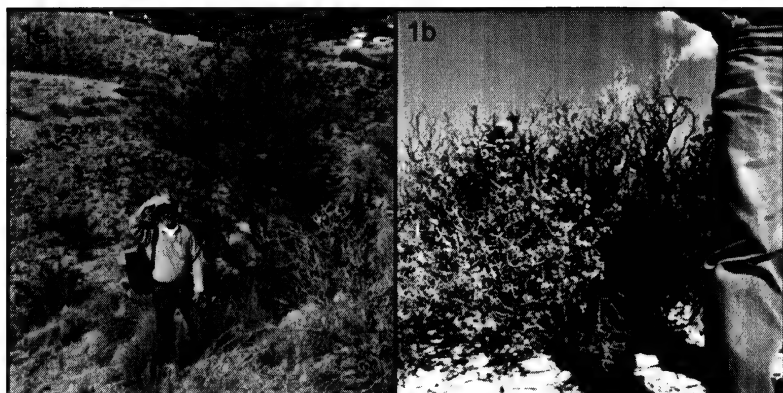
Fig. 1a. The *Quercus hinckleyi* x *vaseyana* hybrid oak with human standing beside it to show the size of the tree. b. Typical motte of *Q. hinckleyi* in January, showing the small stature of the trees.

2a. *Q. hinkleyi*   2b. putative hybrid   2c. *Q. vaseyana*
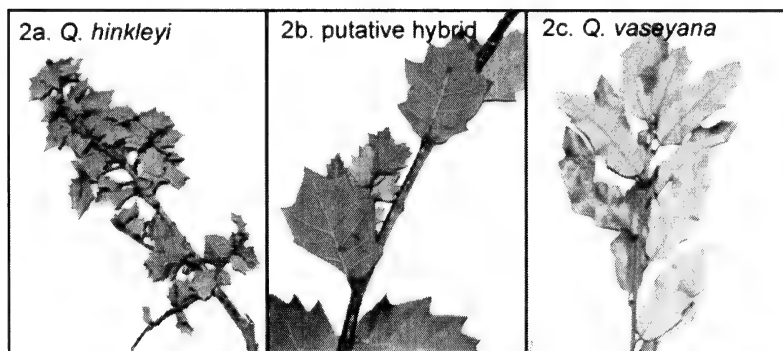
Fig. 2. Leaves of: 2a. *Q. hinckleyi, S. Weyerts 104* (SRSC); 2b. *Q. hinckleyi* x *Q. vaseyana* hybrid, *M. K. Terry 950* (SRSC); 2c. *Q. vaseyana, A. M. Powell & S. Powell 3733* (SRSC).
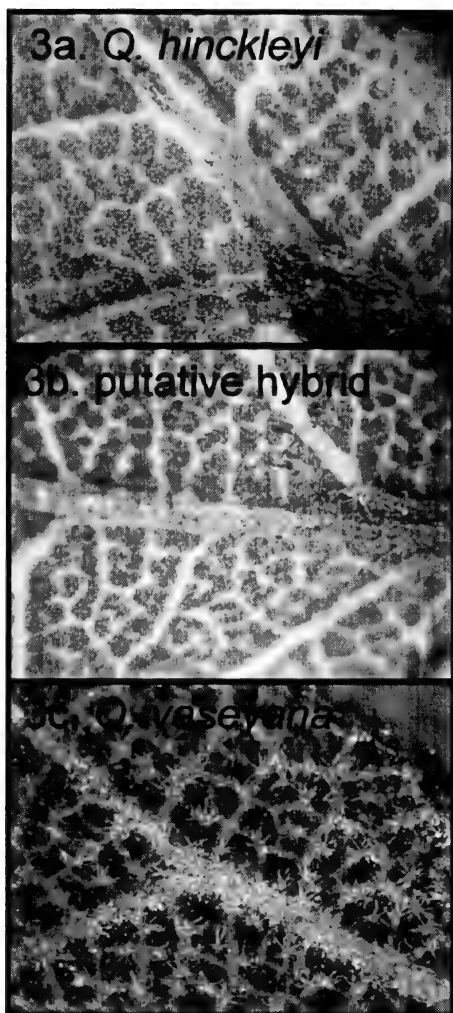
Fig. 3. Abaxial leaf surfaces of: 3a. *Q. hinckleyi* (same specimen as in Fig. 2a), showing absence of stellate pubescence; 3b. putative hybrid of *Q. hinckleyi* x *Q. vaseyana* (same specimen as in Fig. 2b), showing a minimal amount of somewhat malformed stellate pubescence on the veins; 3c. *Q. vaseyana* (same specimen as in Fig. 2c), showing abundant stellate pubescence.

TAXONOMY OF *STEVIA SEEMANNII* AND *S. ALATIPES* (ASTERACEAE) WITH DESCRIPTION OF A NEW SPECIES, *S. ENIGMATICA*, FROM AMONGST THEIR MIDST

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ABSTRACT

The taxonomy of *Stevia seemannii* vis-à-vis *S. alatipes* is discussed in some detail, noting that Grashoff, in his seminal treatment of *Stevia* for Mexico, confused the two names, applying the name *S. seemannii* to distinct populations from southern Mexico that are better treated as the newly proposed **Stevia enigmatica** B.L. Turner, **sp. nov**. The name *S. alatipes* is believed to be a synonym of *S. seemannii*, the type from the Durango-Sinaloa border along Mexican Highway 40. A map showing the distribution of the taxa concerned is provided, along with a photograph of the holotype of *S. enigmatica*. *Phytologia 92(3): 407-412 (December 1, 2010).*

KEY WORDS: Asteraceae, Eupatorieae, *Stevia,* Mexico

Grashoff (1972) provided an exceptional, as yet unpublished, account of the Mexican species of *Stevia*. In this he recognized both *S. alatipes* B.L. Rob. and *S. seemannii* Sch.-Bip. In his discussion of the latter, he noted that it was known with certainty only from Oaxaca and Guatemala and that "Seemann's label data would support that he collected the type in or near Sinaloa where other of his collections with this same data were collected."

McVaugh (1984), in his treatment of *Stevia* for his Flora Novo-Galiciana, further commented that *S. seemannii* was typified by material (holotype, P, examined by both McVaugh and Grashoff) collected by Seemann "from the area where Durango, Sinaloa and Nayarit adjoin." He also called attention to its similarity to *S. alatipes*,

noting that it "differs from the latter in the smaller and more congested inflorescence, in which the longest peduncles are ca 5 mm long or less. The phyllaries are eglandular, ca 7 mm long; the achenes homomorphic, 3-awned, with scale-pappus 0.7 mm long, or in some buds 4- or 5-awned. The relationship to the group of *S. alatipes* needs to be investigated further."

McVaugh summed up the matter, as follows:

Grashoff (1972), like Robinson (1930) before him, did not allude to any similarity between *S. alatipes* and *S. seemannii*, and in fact both authors separate the two rather widely in their arrangements of the genus, basing the separation chiefly on the form of the inflorescence, i.e., in general on the differences set forth in the first lead of the key to *Stevia* in this Flora. According to Grashoff, *S. seemannii* is known from modern collections only from the interior uplands of Guatemala and Oaxaca, and he assumes the type was mistakenly labeled as from the "Sierra Madre." As far as I can ascertain, Seemann did not collect plants in Central America or Mexico between Panama and Mazatlan, so there is evidently some error or misunderstanding involved.

Nevertheless, McVaugh (1984) accepted the taxonomy of Grashoff, relegating *S. seemannii* as one of the "Doubtful" or "Excluded" taxa of *Stevia*.

In my treatment of *Stevia seemannii* for Mexico (Turner 1997) I opined that the species, "as circumscribed by Grashoff, is a discreet morphological entity, well-separated from *S. alatipes* by its shorter, eglanduar peduncles, smaller leaves, etc., and that additional collections will be found between the localities concerned, the widespread-but-uncommon distribution among apomictic species of *Stevia* being a frequent phenomenon."

In preparation of the present paper, I restudied the above problem and conclude that the type of *S. seemannii* is a form of *S. alatipes* having more congested heads and involucres lacking stipitate glands. Similar plants of *S. seemannii* occur elsewhere in Jalisco, if not

Sinaloa, from whence the type. In short, I take *S. seemannii* to be an earlier name for *S. alatipes*.

As noted in the above, Grashoff called attention to the fact that *S. seemannii* is seemingly confined to southern Mexico. The so-identified plants from this region superficially resemble *S. alatipes* (= *S. seemannii*, as defined by Grashoff), but differ in having pubescent roots. Indeed, Grashoff (1972) noted *S. seemannii* (meaning Oaxacan and Central American plants) to be "related to *S. hirsuta* and *S. seemannioides* sharing" the remarkable feature of grey-tomentose roots. All three species have leaves confined to the lower portion of the stem (thus superficially resembling *S. seemannii* of more northern regions). Such roots are not found elsewhere in the genus, to my knowledge; at least they do not occur in populations of their hypothetical relatives from northern Mexico, and it would appear that plants from southern Mexico, referred to as *S. seemannii* by Grashoff (cf. Fig. 2), belong to an alliance of taxa unrelated to *S. seemannnii* proper and are perhaps deserving of a new name, which is provided below:

✓STEVIA ENIGMATICA B.L. Turner, **sp. nov.** Fig 1.

Steviae seemannii Sch.-Bip. similes sed radicibus perspicue pubescentibus, capitulescentiis plus congestis, involucres glandes stipitatas carentibus, et laminis foliorum proximalium plerumque ovatis (vs rotundis).

Perennial herbs 20-60 cm high. **Mid-stems** puberulent to glabrate. **Leaves** 3-6 cm long, 1-3 cm wide, somewhat thickened; petioles 1-3 cm long, sometimes winged; blades ovate, palmately 3-5 nervate from the base, sparsely puberulent below, glabrous above, the margins revolute. **Capitulescence** an array of mostly 1-5 congested heads, the ultimate peduncles mostly 1-5 mm long. **Involucres** mostly 7-9 mm long; bracts puberulous to nearly glabrate, the apices acuminate. **Florets** pink to purple, 6-7 mm long. **Achenes** isomorphic, the body ca 4 mm long; pappus of 3-5 awns, 6-7 mm long, below these a crown of 3-4 scales, 0.8-1.0 mm high. **Chromosome numbers,** $2n = 33, 44, 55$ (Watanabe et al. 2001, as *S. seemannii*).

TYPE: **MEXICO. OAXACA**: "Crest of ridge and steep SW facing slope 90-100 km SW of Sola de Vega along road to Puerto Escondido." 1830 m, 25 Oct 1984, *D.E. Breedlove 62028* (Holotype: TEX)

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. GUERRERO**: "42 km SW from junction with hwy 95, on the way from Chilpancingo to Filo de Caballo," 2080 m, 8 Oct 1997, *Yahara et al. 861* (TEX). **OAXACA**: 34.5 km N from Oaxaca to Valle Nacional along Mex 175, 2300 m, 19 Nov 1997, *Yahara et al. 1079* (TEX). "27.1 km S from JCT on Mex 190, on the way to Santa Maria Ecatepec," 1850 m, 11 Nov 1997, *Yahara et al. 1113, 1114* (TEX). 29.4 km N of Mixtepec, on the way to Oaxaca, 1600 m, 12 Nov 1997, *Yahara et al. 1126* (TEX). 34.8 km N of Juchatengo, on the way from Puerto Escondido to Oaxaca, 2100 m, 13 Nov 1997, *Yahara et al. 1141* (TEX). 3.3 km SW of San Miguel el Grande, 2455 m, 28 Nov 2003, *Yahara et al. 2843* (TEX). 3.5 km NE from JCT in Oaxaca, on the route 175 to Valle Nacional, 2140 m, 16 Nov 1995, *Yahara & Soejima 325* (TEX).

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Fig. 1. *Stevia enigmatica*, holotype.
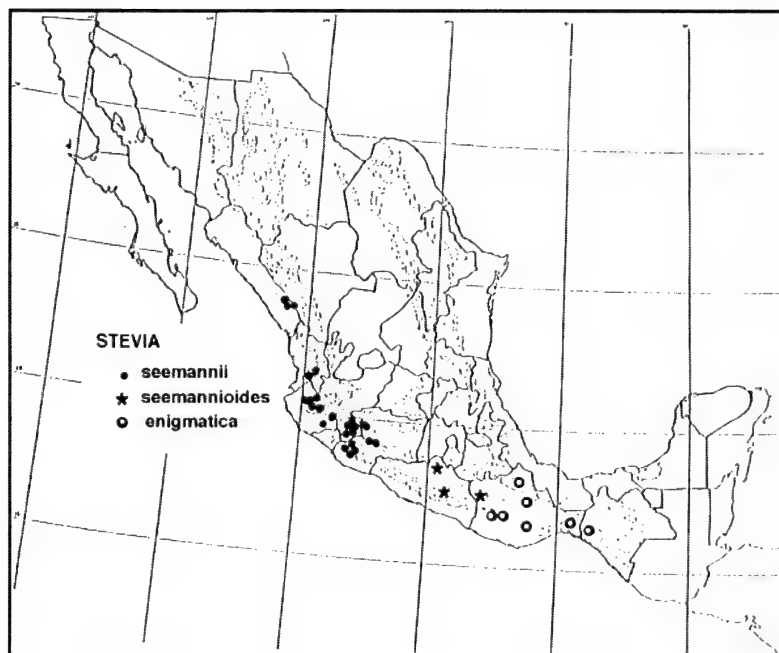
Fig. 2. Distribution of *Stevia* spp.

DISCOVERY OF A NEW POPULATION OF *JUNIPERUS GRACILIOR* VAR. *URBANIANA* FROM THE DOMINICAN REPUBLIC: ANALYSES OF LEAF TERPENOIDS AND SNPS FROM nrDNA AND trnC-trnD

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ABSTRACT

A new population of *Juniperus gracilior* var. *urbaniana* is reported from the Dominican Republic (DR). Previously, the taxon has been known from only one location on the slopes of Pic La Selle, Haiti. The new DR population is not typical of the variety and differs in a few SNPs as well as some differences in its volatile leaf oil. The new population of *J. g.* var. *urbaniana* may be of hybrid origin with *J. g.* var. *ekmanii*. *Phytologia 92(3): 413-423 (December 1, 2010).*

KEY WORDS: *Juniperus gracilior var. urbaniana, J. g. var. gracilior, J. g. var. ekmanii,* Cupressaceae, leaf terpenoids, nrDNA, trnC-D, taxonomy.

Juniperus gracilior var. *urbaniana* (Pilger & Ekman) R. P. Adams is known from only a single population on Pic La Selle, Haiti, where it grows as a prostrate plant in the pine forest on an unusual

white, chalky soil (Adams, 2008). Recently, shrubby junipers were discovered in the Parque Nacional Siera de Bahoruco (Baoruco), Dominican Republic (DR), near the Haitian border. Because the determination of these juniper varieties is very difficult based only on morphology, sequencing of nrDNA and trnC-trnD was performed along with analyses of the volatile leaf oils to more precisely determine the relationship of the shrubby junipers to *J. gracilior var. urbaniana* from Haiti, *J. g.* var. *ekmanii* (Florin) R. P. Adams, Haiti and *J. g.* var. *gracilior* Pilger, Dominican Republic.

MATERIALS AND METHODS

Specimens collected: taxon, acronym, collector number, location: *J. barbadensis* (BA), *Adams 5367-5371;* Petit Piton, St. Lucia, BWI; *J. bermudiana* (BM), *Adams 11080-11082*, Bermuda; *J. gracilior* var. *ekmanii* (EK), *Adams 7653-7654*, 3-4 km ne Mare Rouge, Pic la Selle, Haiti; *J. gracilior* var. *gracilior* (GR), *Adams 7664-7667,* w of Constanza, Dominican Republic, *Adams 3097-3105*, Pedernales , DR; *J. gracilior* var. *urbaniana* (UR) *Adams 7656-7658,* 4-5 km ne Mare Rouge, Pic la Selle, Haiti, *Jimenez 4160*(3), (= *Adams 12005, 12006, 12314* at BAYLU), Parque Nacional Siera de Bahoruco (Baoruco), N 18° 14' 57", W 71 ° 37' 53", 2100 m; *J. lucayana: Adams 5259-5280*, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; *Adams 5281-5282*, Havana Botanical Garden (seed from Isle de Pinos), Cuba; *J. saxicola* (SX) *Adams 5284-5285,* w slope of Pico Turquino, Prov. Granma/ Santiago de Cuba boundary, Cuba; *J. virginiana* var. *virginiana* (VG) *Adams 6753-6755*; on hwy. I35, Hewitt, TX; *J. virginiana* var. *silicicola* (SI) *Adams 9186-9188,* Ft. Desoto Park, Mullet Key, Florida. Herbarium vouchers are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

PCR amplification ITS (nrDNA), trnC-trnD amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (trnC-trnD) or

K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl$_2$ according to the buffer used) 1.8 μM each primer. See Adams, Schwarzbach and Morris (2008) for the nrDNA and trnC-trnD primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (S. San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Compositional analyses of the volatile leaf oils of all the taxa in Hispanola are shown in Table 1. Notice that the oil of the shrub from DR is dominated by bornyl acetate as are all the taxa from DR. The oils of *J. g.* var. *urbaniana* appear to share only a few unique compounds: elemol, unknown 1611, β-eudesmol and α-eudesmol. The oils of *J. g.* var. *ekmanii* and var. *urbaniana* also share some unique compounds: δ-2-carene, isoborneol, germacrene D, piperitone and methyl eugenol. Although some of these are found in trace amounts in the *J. g.* var. *gracilior* plants from Pedernales (gracP in Table 2). The oils of *J. g.* var. *urbaniana* from DR and Haiti also have some quantitative differences: sabinene (4.5, 12.8), borneol (10.7, 1.6) and bornyl acetate (38.1, 26.2). But overall, it appears that the leaf oil of the DR shrubs are most similar to var. *urbaniana* from Haiti. The oil

from putative *J. g.* var. *gracilior* from Pedernales (near the shrubby junipers) is similar to both *J. g.* var. *gracilior*, Constanza and *J. g.* var. *ekmanii* (Table 1) in sharing several unique compounds: unknowns 900 and 907 and linalool. But the oil also has some compounds in common with *J. g.* var. *urbaniana* and *J. g.* var. *ekmanii* (but not *J. g.* var. *gracilior*): trans-sabinene hydrate, citronellol, methyl eugenol, epi-cubebol and trans-cadina-1,4-diene, suggesting the Pedernales population is intermediate between *J. g.* var. *gracilior* and *J. g.* var. *ekmanii*.

Sequencing nrDNA revealed 23 SNPs (Single Nucleotide Polymorphisms) among the Caribbean taxa. A minimum spanning network shows that the DR shrub (Ud, Fig. 1) is separated from *J. g.* var. *urbaniana*, Haiti (Uh, Fig. 1) by 2 SNP differences. Notice that *J.*
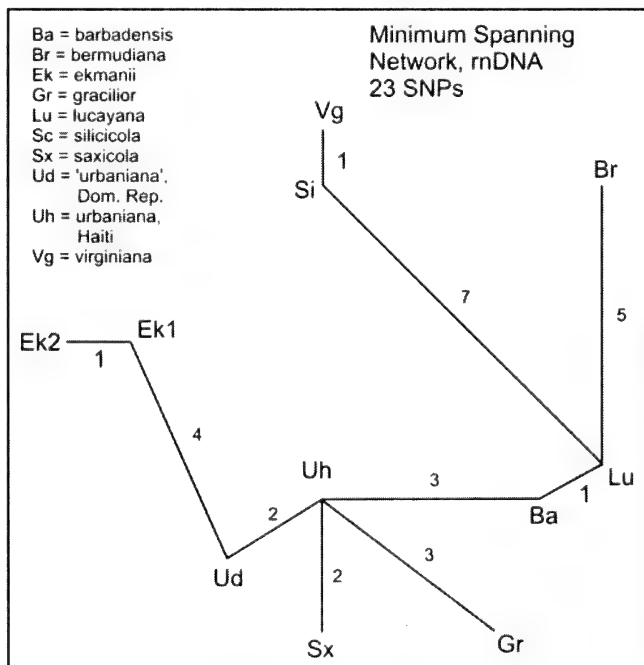
Figure 1. Minimum spanning network using 23 nrDNA SNPs for the Caribbean junipers.

saxicola is linked by 2 SNPs (Fig. 1) to *J. g.* var. *urbaniana*, Haiti (Uh, Fig. 1). It is interesting that Ud is intermediate to *J. ekmanii* (Ek1, Ek2).

Analyses of trnC-trnD sequences revealed no differences between the DR shrub and *J. g.* var. *gracilior*, and one difference between it and *J. g.* var. *urbaniana* (Haiti) and *J. g. ekmanii* (Fig. 2). Integrating these data with previous data (Adams, et al. 2008) shows (Fig. 2) that the 'urbaniana' shrubs from Dominican Republic are in the group with *J. g.* var. *gracilior*, whereas *J. g.* var. *urbaniana* (Haiti) is in a group with *J. g.* var. *ekmanii* and *J. saxicola*. However, these groups are separated by only one SNP.
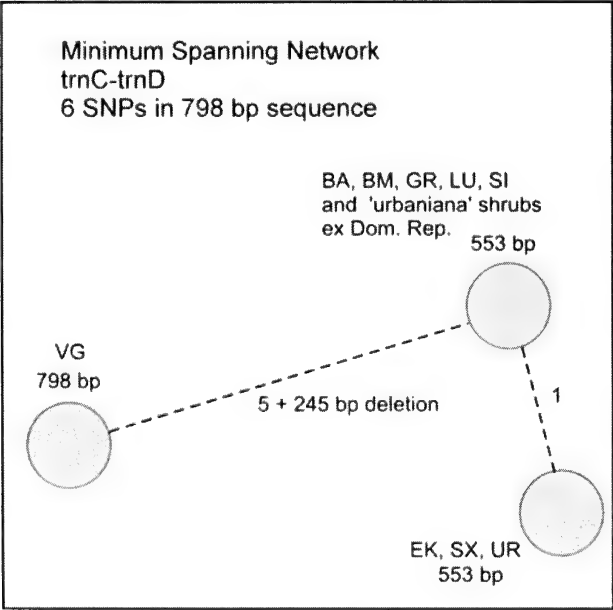
Figure 2. Minimum Spanning Network based on 6 SNPs from trnC-trnD 798 bp sequence.

CONCLUSIONS

It is noteworthy that a new population of *J. g.* var. *urbaniana* has been discovered. This new site is in the same mountain range as Pic La Selle and represents an eastern extension of the range (Fig. 3). The new population differs somewhat from the Haiti plants in that these shrubs are about 0.3 m tall x 1-2 m wide, whereas the plants in Haiti are prostrate (5-10 cm tall x 3-5 m wide). The DR shrubs appear to grow on a hard-pan type of soil. The DR plants differ somewhat in their DNA sequences and volatile leaf oils that suggests possible hybridization with *J. g.* var. *ekmanii*. Nevertheless, it is still useful for conservation purposes to have this new population, because the Haitian population is very small and certainly threatened.
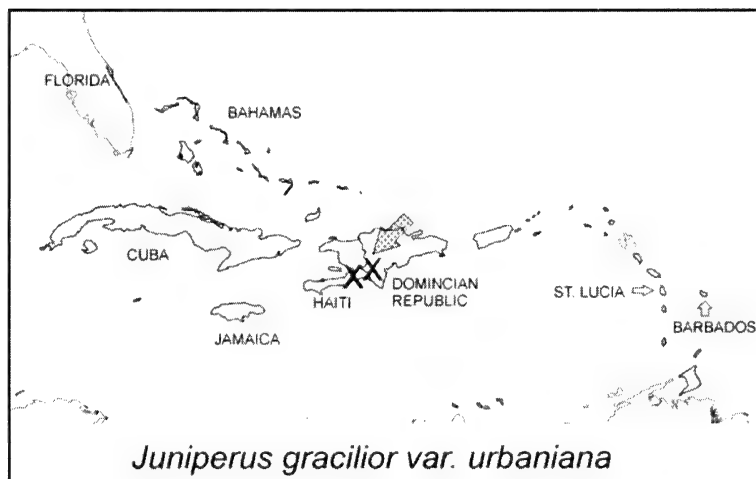
Figure 3. Distribution map for *Juniperus gracilior* var. *urbaniana*.

ACKNOWLEDGMENTS

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Table 1. Comparisons of the volatile leaf oils of *J. gracilior* var. *urbaniana*, shrub from Dominican Republic (urbD), *J. g.* var. *urbaniana*, Haiti (urbH), *J. g.* var. *ekmanii* (ekman), *J. g.* var. *gracilior*, Constanza, DR (gracC) and *J. g.* var. *gracilior*, Pedernales , DR (gracP).   Compounds in bold appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.   RI is the Kovat's Index using a linear approximation on DB-5 column.

RI	compound	urbD	urbH	ekman	gracC	gracP
900	**unknown, 43,55,125,140**	-	-	t	**0.2**	**0.3**
907	**unknown, 43,55,125,140**	-	-	**t**	**0.2**	**0.3**
921	tricyclene	1.9	1.0	1.3	3.2	0.9
924	α-thujene	0.4	0.6	0.3	0.7	0.7
932	α-pinene	3.0	1.6	3.3	3.7	2.1
946	camphene	1.9	1.2	1.5	2.5	1.2
969	sabinene	4.5	12.8	10.1	4.4	11.8
974	β-pinene	0.2	0.4	0.4	.04	0.3
988	myrcene	2.6	3.2	2.5	2.2	3.0
1001	**δ-2-carene**	**0.2**	**0.6**	**0.2**	-	-
1002	α-phellandrene	0.1	t	t	t	0.1
1008	δ-3-carene	-	-	-	t	-
1014	α-terpinene	0.9	2.4	0.7	1.1	1.3
1020	p-cymene	1.4	2.8	0.4	2.6	1.0
1024	limonene	6.5	9.0	8.4	7.9	7.0
1025	β-phellandrene	3.0	5.0	4.2	3.8	3.6
1054	γ-terpinene	2.0	2.8	1.4	3.3	2.6
1065	cis-sabinene hydrate	0.5	1.0	0.6	0.1	0.8
1086	terpinolene	0.6	1.0	0.5	0.6	0.7
1090	6,7-epoxymyrcene	-	t	t	t	0.1
1098	trans-sabinene hydrate	0.3	0.4	0.3	-	0.4
1098	**linalool**	**t**	-	**0.6**	**0.1**	**0.6**
1100	n-nonanal	t	t	-	-	-
1112	trans-thujone	0.1	0.1	0.7	0.6	0.2
1118	cis-p-menth-2-en-1-ol	0.3	0.4	0.4	0.2	0.5
1118	cis-limonene oxide	-	-	0.1	-	-
1136	trans-p-menth-2-en-1-ol	0.1	0.4	0.1	0.1	0.4
1141	camphor	1.2	1.6	3.4	0.7	0.8

RI	compound	urbD	urbH	ekman	gracC	gracP
1145	camphene hydrate	1.0	0.6	1.3	0.4	0.6
1154	**karahanaenone**	-	-	-	**0.1**	-
1155	**isoborneol**	**0.2**	**0.2**	**0.1**	-	**t**
1165	**borneol**	**10.7**	**1.6**	**3.3**	**1.1**	**1.7**
1167	umbellulone	0.2	t	t	t	t
1174	terpinen-4-ol	3.2	4.8	3.7	3.3	7.4
1178	naphthalene	-	0.2	-	-	-
1184	dill ether	-	-	0.1	0.2	0.3
1186	**α-terpineol**	**1.4**	**1.0**	**0.3**	**0.2**	**0.4**
1191	cis-dihydrocarvone	-	-	t	t	-
1195	cis-piperitol	-	-	-	t	-
1195	methyl chavicol	-	t	-	-	0.3
1200	trans-dihydrocarvone	-	-	t	-	-
1207	trans-piperitol	-	t	-	-	0.5
1223	citronellol	-	-	0.3	-	0.4
1232	thymol, methyl ether	-	-	-	t	-
1239	carvone	t	-	t	t	t
1241	carvacrol, methyl ether	-	-	-	-	-
1249	**piperitone**	**0.8**	**0.8**	**0.3**	-	**t**
1274	pregeijerene B	-	0.2	-	-	-
1284	bornyl acetate	38.1	26.2	37.6	46.2	33.0
1287	**safrole**	-	-	-	-	**2.6**
1298	carvacrol	-	-	-	r	-
1299	terpin-4-yl acetate	t	-	-	-	t
1326	iso-dihydrocarvyl acetate	-	-	-	0.1	-
1345	α-cubebene	0.1	-	-	-	-
1356	neo-iso-dihydrocarvyl acetate	-	-	-	0.2	-
1365	cis-carvyl acetate	-	-	-	0.1	-
1374	α-copaene	0.2	-	-	-	-
1380	daucene	-	-	0.2	-	-
1387	β-bourbonene	t	-	-	-	-
1387	β-cubebene	0.1	-	t	-	-
1396	duvalene acetate	-	-	-	-	t
1403	**methyl eugenol**	**1.2**	**0.6**	**0.1**	-	**2.2**
1401	α-cedrene	-	-	t	-	-
1413	β-funebrene	-	-	t	-	-

RI	compound	urbD	urbH	ekman	gracC	gracP
1417	(E)-caryophyllene	0.3	t	t	0.4	-
1429	cis-thujopsene	-	-	t	0.1	-
1448	**cis-muurola-3,5-diene**	**0.4**	**0.2**	**0.1**	**0.1**	**0.1**
1452	α-humulene	0.2	t	-	t	-
1475	trans-cadina-1(6),4-diene	0.4	0.2	0.1	0.2	0.1
1478	γ-muurolene	-	-	t	t	-
1480	**germacrene D**	**0.5**	**0.2**	**t**	**-**	**-**
1493	**trans-muurola-4(14),5-diene**	**1.1**	**0.6**	**0.1**	**0.2**	**0.4**
1493	epi-cubebol	0.1	t	0.1	-	0.2
1500	α-muurolene	0.2	t	0.1	t	-
1513	cubebol	0.4	0.4	0.6	t	0.5
1522	δ-cadinene	0.9	0.4	0.6	0.5	0.3
1528	zonarene	0.3	0.2	0.1	0.2	0.1
1530	dauca-4(11),8-diene	-	-	t	-	-
1532	γ-cuparene	-	-	-	t	-
1533	trans-cadina-1,4-diene	0.1	t	t	-	t
1537	α-cadinene	-	-	t	-	-
1544	α-calacorene	-	-	-	t	-
1548	**elemol**	**0.1**	**0.8**	**-**	**-**	**-**
1555	**elemicin**	**-**	**t**	**t**	**0.4**	**2.5**
1574	**germacrene-D-4-ol**	**-**	**-**	**0.8**	**-**	**-**
1582	caryophyllene oxide	t	-	t	0.1	0.2
1587	trans-muurol-5-en-4-α-ol	t	t	t	-	t
1599	widdrol	-	-	t	t	-
1600	**cedrol**	**-**	**t**	**0.3**	**0.2**	**-**
1607	β-oplopenone	t	-	0.3	-	t
1608	humulene epoxide II	-	-	-	t	t
1611	**unknown,43,109,119,254?**	**0.3**	**0.6**	**-**	**-**	**-**
1627	1-epi-cubenol	0.5	0.6	0.4	0.5	0.6
1630	γ-eudesmol	-	0.1	-	-	-
1638	epi-α-cadinol	t	t	0.2	0.1	-
1638	epi-α-muurolol	t	t	0.2	0.1	-
1644	α-muurolol	t	t	t	t	-
1645	cubenol	-	-	-	-	0.2
1649	**β-eudesmol**	**t**	**0.4**	**-**	**-**	**-**
1652	**α-eudesmol**	**t**	**0.4**	**-**	**-**	**-**

RI	compound	urbD	urbH	ekman	gracC	gracP
1652	**α-cadinol**	-	-	0.4	-	-
1685	germacra-4(15),5,10(14)-trien-1-al	t	t	t	t	0.3
1887	oplopanonyl acetate	-	-	t	-	-
1905	isopimara-9(11),15-diene	t	-	-	-	t
2055	abietatriene	t	t	-	-	t
2087	abietadiene	-	-	-	-	0.1
2282	**sempervirol**	**0.3**	**0.4**	-	**0.2**	**0.6**
2314	trans-totarol	0.3	0.4	0.4	2.0	0.5
2331	trans-ferruginol	t	t	-	0.1	t

SEED DISPERSAL IN *JUNIPERUS*: A REVIEW

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ABSTRACT

The dispersal of *Juniperus* seeds is reviewed. Although birds
are the major dispersal agents, mammals can be significant, but even
harvester ants have been found to carry berries up to 15 m to their nests,
then remove the pulp and deposit the seeds outside their mounds.
Phytologia 92(3): 424-434 (December 1, 2010).

KEY WORDS: Seed dispersal, *Juniperus*, birds, mammals, harvester
ants, germination.

The seminal paper in juniper seed dispersal was by Phillips
(1910). His oft-cited paper was based on six years of field observations
and interviews with naturalists concerning the dispersal of seeds of
several *Juniperus* species. Phillips noted that because many juniper
species' berries (female cones) mature in the fall and stay on the tree
until spring, these present birds with a high energy food source when
other seeds may be in short supply. In addition, the berries are often
blue with a white bloom that makes them discernible even at
considerable distances (Phillips 1910, Adams 2008). Phillips (1910)
notes that E. A. Mears fed caged Bohemian waxwings (*Ampelis
garrulous*) berries of *J. scopulorum* Sarg. and observed that over 900
berries passed through the birds in 5 hours. Clearly, these birds can
consume and rapidly spread juniper seed. Phillips noted that a flock of
50 robins consumed all of the fruits on a juniper tree in a single day!

He also noted the occurrence of 'fence row' junipers that were presumably 'planted' by birds resting on fences.

Phillips (1910) compiled a list of birds that had been found to consume juniper berries (Table 1). In addition, he reported that in Texas, the feces of raccoons, foxes, wildcats and chipmunks contained large amounts of juniper seeds from November to March. But he concluded that mammals are of rather minor importance in juniper-seed dispersal, compared to birds. Phillips also gave an interesting account of a Texas cattle herd that was driven to Kansas and grazed a few days on the treeless prairie, that later gave rise to a small juniper population.

Table 1. Birds that have been found to consume juniper berries (Phillips, 1910).

Juniperus species
Canachites canadensis - Canada Grouse
Corvus brachyrhynchos - Common Crow
Empidonax trailli - Traill Flycatcher
Oreortyx p. plumiferus - Plumed Quail
Pedioecetes phasianellus - Sharp-tailed Grouse

Juniperus communis
Merula migratoria - Robin
Parus atricapillus - Black-capped Chickadee

Juniperus sabina
Tyrannus tyrannus - Eastern Kingbird

Juniperus scopulorum
Ampelis garrulous - Bohemian Waxwing

Juniperus utahensis (J. osteosperma)
Meleagris gallopavo - Wild Turkey

Juniperus virginiana
Ampelis cedrorum - Cedar Waxwing
Carpodacus purpureus - Purple Finch
Corvus brachyrhynchos - Common Crow
Colaptes auratus - Yellow-shafter Flicker
Dendroica coronata - Myrtle Warbler
Dryobates pubescens - Downy Woodpecker
Hesperiphona vespertina - Evening Grosbeak
Hylocichla guttala -Hermit Thrush
Lagopus leucurus - White-tailed Ptarmigan
Merula migratoria - Robin
Mimus polyglottos - Mockingbird
Passerella iliaca - Fox Sparrow
Pinicola enucleator - Pine Grosbeak
Sayornis phoebe - Say's Phoebe
Sialia sialis - Bluebird
Sphyrapicus varius - Yellow-bellied Sapsucker
Tyrannus tyrannus - Kingbird

McAtee (1947) published an exhaustive annotated bibliography of papers dealing with the distribution of seeds by birds and noted that shade-tolerant cedars often develop under "perch trees."

Abbott and Belig (1961) found that red squirrels fed on *J. communis* berries during the winter, but avoided the prickly foliage of common juniper.

Poddar and Lederer (1982) reported that Townsend's solitaires feed exclusively on berries of *J. occidentalis* during the winter. They analyzed the nutritional content and found 4% protein, 16 % lipid and 46% carbohydrate and concluded that *J. occidentalis* berries provide sufficient nutrients and energy to sustain solitaires during the winter.

One of the most detailed studies of the fate of juniper berries was conducted by Holthuijzen and colleagues (overview and references in Holthuijzen, Sharik and Fraser, 1987). Figure 1 shows a summary of their results. Noteworthy is that 65.5% of the berries were dispersed
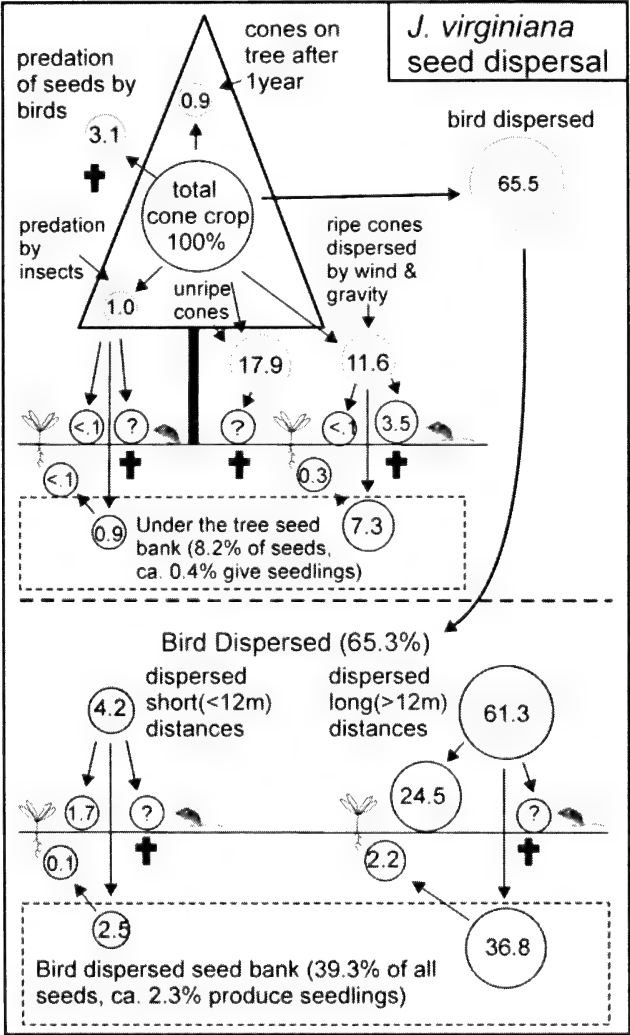
Figure 1.   Dispersal of seeds in *Juniperus virginiana* (adapted from Holthuijzen et al. 1987).

by birds (Fig. 1). In addition, a large proportion (17.9%) of the cone-crop fell as unripe cones. This appears to be a common situation as I (RPA) have observed many unripe berries under various juniper species. However, very few of the seeds deposited under the tree canopy produce seedlings (ca. 0.4% of the seed crop, Holthuijzen et al. 1987). This seems to imply that the allelopathic nature of juniper foliage may inhibit seed germination under the canopy. Of course, shading and competition for moisture are additional factors.

Of the 65.5% berries dispersed by birds, about 93.6% were dispersed greater than 12 m from the tree. Of the 2.7% that produce seedlings, about 85% of these arose from long distance dispersal of seeds. Of course, a considerable number of seeds are deposited on the soil surface (1.7%, 24.5%) and these may germinate in favorable years.

Holthuijzen and Sharik (1985) showed that seed dispersal and seedling patterns in *J. monosperma* had very definite effects under the tree canopies (Fig. 2). The great majority of seeds fall under the tree, whereas, the majority of the seedlings are near, but outside the canopy of the tree (Fig. 2). Again, this may reflect allelopathy and/ or the shade and moisture constraints under the tree canopy.
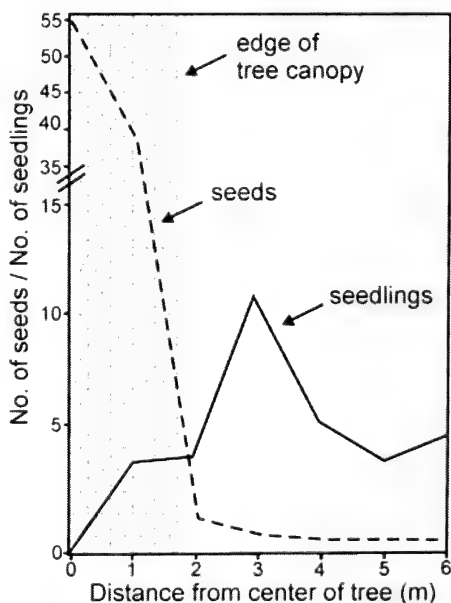
Figure 2. Number of seeds (per 0.1 m^2) vs. seedlings (adapted from Holthuijzen and Sharik 1985).

The passage of juniper seeds through the digestive tract of Cedar Waxwings was examined by Holthuijzen and Adkisson (1984) who reported an average residence time of only 12 mins. At the rate of feeding, they estimated that a Cedar Waxwing would eat about 53 berries per hour. So it is easy to see that Cedar Waxwings feeding in a large *J. virginiana* tree could deposit many of the seeds under the tree canopy, as shown in Fig. 2.

Holthuijzen and Sharik (1985) found that seeds collected from the feces of warbler and waxwing germinate at a rate of 55.0% and 27.6%, compared to the control (16.1%) germination tests. It is thought that the passage through the digestive tract scarifies the seeds making it easier to absorb water.   However, Salomonson (1978) fed *J. monosperma berries* to Townsend's solitaires and then germinated the seeds and got mixed results. Berries collected in March, 1974 and fed to the birds had a germination rate of 24% vs. the control germination rate of 18%. In contrast, berries collected in March, 1974, then stored in dry, dark boxes at 21°C until November, 1974 (6 mos.) displayed the reverse pattern: those fed germinated at 35% vs. 45% for the control.

Johnsen (1962) examined the effects of seed passage through various animals and germination for *J. monosperma* seeds. Table 2 shows that digestion by animals speeds up the germination, but did not appear to increase the percentage of germination. It is assumed that the control germination seeds were kept moist for the entire 10 weeks. In northern Arizona, it would be very unlikely that soils might be kept moist for 10 weeks, so rapid germination following a rain would appear to be favored (i.e., favoring animal excreted seeds).

In the Canary Islands, *Juniperus cedrus* is dispersed by thrushes, ravens and lizards (B. Rumeu, pers. comm.).   Control germination reached about 21% (after 200 days), whereas seeds passed by ravens and thrush attained about 33% germination. In contrast, seeds passed through the digestion tract of lizards showed reduced germination of about 10% (B. Rumeu, pers. comm.).   Rumeu et al. (2009) reported that during the winter, they obtain about 98% of their diet from *J. cedrus* berries.

Table 2. Effects of passage through the digestive tracts of various animals on the germination of *J. monosperma*. Data from Johnsen (1962).

animal	cumulative % germination by weeks									
	1	2	3	4	5	6	7	8	9	10
none (control)	0	1	6	16	29	29	36	41	44	44
bird	11	15	20	39	43	43	44	44	44	44
coyote or fox	2	10	28	37	40	44	45	45	45	45
packrat	8	14	22	40	44	44	45	45	45	45
jackrabbit	7	13	25	45	50	51	51	51	51	51
sheep	3	12	31	42	45	46	46	46	46	46

Schupp et al. (1997) examined feces of Nuttall's cottontail rabbits, mule deer, elk and coyotes for the presence of *J. occidentalis* seeds. Table 3 shows that, in general, these mammals do not consume vast quantities of juniper berries, except for coyotes that have a significant amount of seeds in some scats. We have observed *J. osteosperma* seeds in coyote scat in Arizona (Fig. 3), where it makes up the major portion of the scat.

Figure 3.   *Juniperus osteosperma* seeds in coyote scat (near Cottonwood, AZ)

Table 3. Numbers and frequency of *J. occidentalis* seeds in defecations of 4 mammals.

	# pellets or scats	# with seeds	total # seeds
cottontail	2046 pellets	8	8
mule deer	19,414 pellets	6	6
elk	562 pellets	0	0
coyote	29 scats	4	437

Horncastle and Hellgren (2004) reported opossums and deer mice removed *J. virginiana* berries from beneath trees. They remarked that rodents appear to be seed predators and are not effective in dispersal.

Chavez-Ramirez and Slack (1993) analyzed scat from four carnivores (raccoon, ringtail, and brown and gray foxes. They found that during the winter months when *J. ashei* fruits are plentiful, all of these carnivores consumed considerable amounts of the berries. Because these carnivores have large home ranges (3.2 km to 10.4 km) they might be effective in long range dispersal. However, Chavez-Ramirez and Slack (1993) noted that, in at least 15 cases, rodents had consumed as much as 50% of the *J. ashei* seeds in mammal feces.

Harvester ants (*Pogonomyrmex* sp.) are well known to collect seeds and plant materials (Rissing, 1988). In a study at the Desert Botanica Garden, Phoenix, the latter workers found that the diet of *P. rugosus* diet consisted of 87% seeds from three annual plant species. The harvester ant's range varied from 12.7 to 22.1 m (avg. +/- 4 m, 1 SD) over a 3 year period. The larger range (22.1 m) occurred in 1984 when a drought restricted the growth of the favored annual species.

Recently we (DT) observed harvester ants carrying berries of *J. arizonica* from a tree about 15 m from their nest/ mound (Fig. 4). A few cleaned seeds were found next to the mound and one clean seed was found in the nest (fig. 5).
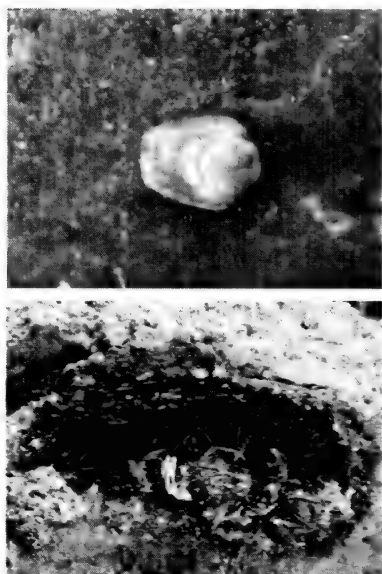
Figure 4. Harvester ant carrying a juniper fruit to its nest/ mound.

Figure 5. (top) cleaned *J .arizonica* seed taken from nest.
(bottom) Fungal farm in ant nest.

      In our situation the ants generally become active in the morning, forming only a single marching column about eight inches wide to the juniper tree. They harvested the berries from the ground only, and returned at a rate of one berry every two minutes. This continued all day (~8 hr) for a week. At such a rate as many as 1600 berries may have been carried to their nest. During this period, the column from the juniper tree to the nest carried only juniper seeds. A few ants, not in this column, cut up leaves from a *Penstemon* near the hole. On another occasion, an ant column was harvesting only bird seed in a bird feeding area. When juniper berries were place along the 'bird seed' column, the ants refused to pick up any berries. It appears that the ants just harvest one kind of material at a time.

        In summary, it appears that harvester ants bring juniper berries into their nest, eat off the pulp and then carry the seeds out to the edge of their mound.

        This is not the first report of harvester ants collecting juniper berries.   Willard and Crowell (1965) reported that *Pogonomyrmex owyheei* carried juniper (*J. occidentalis*) twigs and berries to their nests.

        Although harvester ants may play a role in the dispersal of *J. arizonica* seeds, there is little doubt that birds are the main dispersal agents of seeds of that taxon as well as nearly all *Juniperus* species (Holthuijzen et al. 1987). In fact, Santos et al. (1999) found that birds were about 30 times as effective as mammals in the dispersal of *J. thurifera* seeds in Spain.

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A NEW SPECIES OF *CHAMAESARACHA* (SOLANACEAE) FROM MEXICO AND THE SEPARATION OF *C. CRENATA* FROM *C. VILLOSA*

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ABSTRACT

Prior to a more comprehensive treatment of *Chamaesaracha*, a new species, *C. geohintonii,* a gypsophile from Nuevo León, Mexico is described. The species is most closely related to *C. crenata* and *C. villosa*. The latter two taxa are often difficult to distinguish and some workers consider these to be synonymous (Henrickson 2009). The salient features separating these several taxa are discussed. *Phytologia 92(3): 435-441 (December 1, 2010).*

KEY WORDS: *Chamaesaracha*, Mexico, Texas, Solanaceae

Examination of Mexican collections of *Chamaesaracha,* especially from around Parras, Coahuila and western Nuevo León, have provided additional insight into the status of *C. crenata*, *C. villosa* and an undescribed taxon herein recognized as *C. geohintonii.* The latter is most closely related to *C. crenata* but is smaller in habit and has a different vesture.

Chamaesaracha geohintonii Averett & B.L. Turner, **sp. nov.**
Herba perennis, caulis 15-20 cm alta, pubescentia; folia petiolata, 1.8-3.0 cm longa, lamina rhombica vel ovata, crenata vel undulata. *Chamaesaracha crenata* similis.
TYPE: **MEXICO. Nuevo León, Mpio. Mina**, W of Los Molina, ca 26.04074 N, 100.44492 W, 943 m, "Gypsum hillside." 23 Jul 2007, *G.B. Hinton et al. 28619* (Holotype: TEX-LL).

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Perennial herbs, branching from the base, ascending to nearly upright; vestiture of whitish, mostly simple, trichomes largely lacking glandular hairs; leaves 1-3(4) cm long, lamina ovate to rhombic, crenate to undulate, ca 2/3 the length of the leaf, apices typically obtuse to rounded, bases acute to shortly attenuate; flowers 1-2 from axils, corolla yellow-green, rotate, 1.0-1.5 cm across, calyces, 3-5 mm long, fruit a green berry ca 1 cm in diameter, pericarp thin, dry; seeds ca 25, reniform, rugose-reticulate, brown, 1.5-2.0 mm long.

The species is named for George B. Hinton, avid collector of the flora of north-central Mexico. Familiar with the area concerned, he called attention to the novelty, suggesting that it might be new.

Chamaesaracha geohintonii is closely related to *C. crenata;* the latter occurs principally in Coahuila, but populations are found along the Rio Grande River in southern Brewster Co. and adjacent Presidio Co. in the Big Bend Region of Texas; *C. crenata* is also close to *C. villosa*, which was originally described from Coahuila but also occurs in Chihuahua, Durango and Texas. Scudday (1965) first noted the presence of *C. villosa* in Texas.

Chamaesaracha geohintonii is known only from the type locality, which is northwest of Saltillo, where it occurs in gypsum soils, the site of several localized edaphic endemics (cf. Turner 2010). The distribution of the three species is shown in Figure 3.

Rydberg (1896) described *C. villosa* from near Saltillo, Mexico and *C. crenata* from Parras, ca 110 mi west of Saltillo. All areas of investigation indicate that *C. crenata* is closely related to *C. villosa* and, as already noted (Henrickson 2009), the two are frequently difficult to distinguish. As a part of his early graduate work, Averett (1967) suggested that the two were quite close and "if salient differences exist," they are not clear" but he made no taxonomic changes. Averett (1973) continued to recognize both species; he noted, however, that "the decision for varietal or specific status is somewhat arbitrary in this instance," but at the extremities of their ranges the taxa are relatively distinct and can be recognized by differences in leaf shape and pubescence, as noted in his key and in the original descriptions by Rydberg (1896), characters equivalent to those distinguishing other

species of the genus. Examination of previously unseen Mexican collections of *Chamaesaracha,* from Parras, Coahuila and those of western Nuevo León, and the reexamination of older material from that area, have provided additional insight into the *C. crenata-C. villosa* complex and strengthens the case for maintaining the two as separate species, as suggested herein.

In previous and subsequent studies, Averett (1973, unpubl.) found the type specimens of *C. crenata* and *C. villosa* to be distinct, as were other populations both in Mexico and Texas. For example, all of the populations from about Lajitas, Texas, northwest along the Rio Grande to Hudspeth Co., clearly compare to *C. villosa,* as do populations several kilometers south of Ojinaga, Mexico (e.g., *Averett 156, 157, 158*; *Averett & Powell 184*). There are a few relatively clear populations of *C. crenata* in Big Bend National Park (*Averett & Sikes 238; Warnock 13909; Warnock & Johnston 15909*). Populations in Mexico are mostly distinct, especially in and around the type localities.

Early in his studies, Averett suspected hybridization to be responsible for some of the overlap in characters between the two taxa. Plants obtained from three populations (*Averett & Powell 217, 218, 219*), all within 1 km of each other along the Rio Grande River, southeast of Lajitas, Texas on what is locally known as Big Hill, showed some evidence of hybridizaton, one plant comparing more to *C. crenata*, another to *C. villosa*, and one intermediate. Flavonoid analyses (unpublished) were done on these and other populations of the complex. Similar flavonoid profiles were found in the two species but, interestingly, some additional, highly methylated compounds, were found in the suspected hybrids. These might represent "hybrid compounds" similar to those found in *Baptisia* (Alston et al., 1965) but do not provide conclusive evidence of hybridization.

Another problematic area is to the north and east of Big Bend National Park, east to about Val Verde Co.; in this region there are some exceptionally robust plants in the Black Gap area, just north of the Park, that might be confused with *C. crenata* but otherwise are like *C. coniodes* and possess tetraploid chromosome counts of $n = 24$. Both *C. crenata* and *C. villosa* are diploid with $n = 12$. I now think that most, if not all, of those populations north and east of Brewster Co. in Texas, previously identified as *C. crenata,* are more likely *C. coniodes*.

Since the two taxa are distinct in and around their type localities and in all but a few populations in Trans-Pecos, Texas, I believe the continued recognition of *C. crenata* and *C. villosa* is warranted. *Chamaesaracha geohintonii,* because of its unique vestiture, small leaves, small habit, and more eastern distribution, is not likely to be confused with *C. crenata* or *C. villosa*, nor with other likely relatives such as *C. sordida*. A comparison of the three species is found in Table I. Distributions of the three species, and drawings noting leaf-shape and trichomes are presented in Fig. 1. Photographs of the types of *C. crenata* and *C. villosa* from the Smithsonian Type Specimen Registry can be found online (http://botany.si.edu/types/).

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Table 1. A comparison of three species of *Chamaesaracha*.

	C. crenata	*C. villosa*	*C. geohintonii*
Habit	Robust , stems ascending, 15-40 cm long	Robust , stems ascending, 15-40 cm long	Small plants, stems ascending to upright, usually 15 cm or less
Habitat and distribution	Desert soils, principally in Coahuila	Desert soils, principally in Coahuila and Chihuahua	Gypsum soils in Nuevo León
Pubescence	Long trichomes with an understory of glandular hairs	Villous with long, often forked trichomes, glandular hairs largely lacking	Long unbranched trichomes, glandular hairs largely lacking
Leaves	Petiolate, 4-6 cm long, lamina ovate to rhombic with margins crenate-undulate, ca ¾ the length	Sessile to subsessile, 4-6 cm long, lamina oblong, ovate or rhombic, margins entire to dentate	Petiolate, 1-3 (-4) cm long, lamina ovate to rhombic, margins crenate-undulate, 2/3 the length
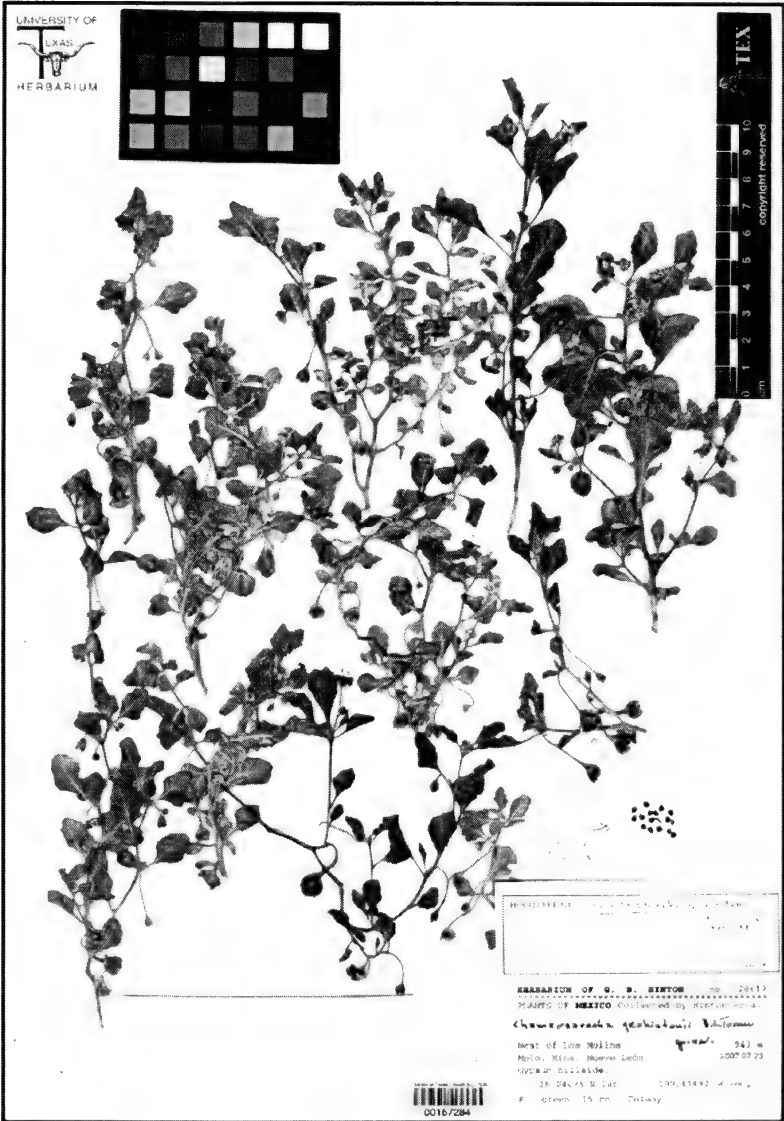
Fig. 1. *Chamaesaracha geohintonii* (Holotype: TEX).
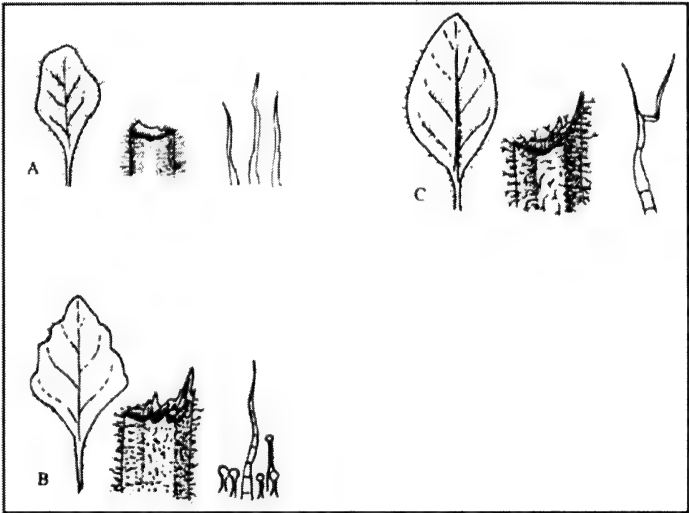
Fig. 2. Leaf outlines, stem, and pubescence types: *Chamaesaracha geohintonii* (A), *C. crenata* (B*)*, and *C. villosa* (C*)*.
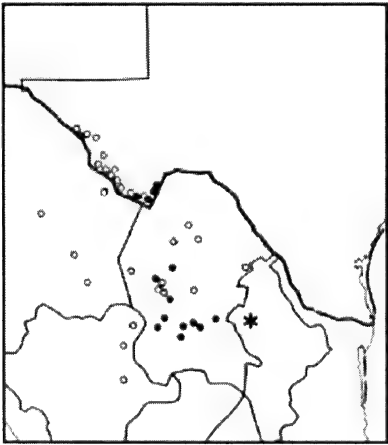
Fig. 3. Distribution of: *Chamaesaracha crenata* (closed circles), *C. villosa* (open circles) and *C. geohintonii* (asterisk).

KEYS TO THE FLORA OF FLORIDA - 26, *CENCHRUS* (GRAMINEAE)

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ABSTRACT

Cenchrus (Gramineae) is represented in Florida by 8 species. All appear to be native, with *C. gracillimus* effectively endemic. The *C. incertus* complex is treated as of three recognizable species, but with significant questions unanswered. Range of *C. tribuloides* is restricted to northern Florida, with plants of the southern peninsula bearing that name being the little-recognized *C. bambusoides*. Nomenclatural history relating to *C. caroliniana* is reviewed. The early but unidentifiable name *C. spinifex* is rejected. One species reported for Florida is excluded. An amplified key is given to the Florida taxa. *Phytologia 92(3): 442-450 (December 1, 2010).*

KEY WORDS: *Cenchrus*, Gramineae, Florida flora.

"Rating weeds in order of badness, I would give the Sandspurs the first place. They are bitter grasses eaten only as a last resort by cattle, and all other weeds in the State combined do not cause as much pain, profanity and danger to life, as these worthless grasses."
              J. C. Neal.   Florida Agricultural Experiment
                 Station, 2nd. annual report.   1890.

        Botanical purists have for generations attempted to lead the public into calling species of *Cenchrus* (Gramineae) either "sand-burs" or "bur-grasses." But with the unlettered common man, they are invariably known as "sandspurs." Here, an effort is made to guide

needful persons to the correct scientific names of these "worthless grasses."

The most recent -- and thus the most predictably followed -- treatment of large scope was a world monograph of *Cenchrus* by Donald G. DeLisle (Iowa State Jour. of Science 37: 259-351. 1963). His work has tended to eclipse the findings of an earlier, admirable study of North American species by Agnes Chase (Contr. U.S. Nat. Herb. 22: 45-77. 1920). Two regional but significant studies were by Lloyd H. Shinners (Rhodora 56: 35. 1954; Field & Lab. 24: 73-74. 1956) of Texan species, and Jose A. Caro & Evangelina Sanchez (Kurtziana 4: 39-50; 95-129. 1967) of (mostly) Argentine species. American species have again been treated by M. T. Stieber & J. K. Wipff (Flora N. Amer. 25: 529-536. 2003). Since the *Cenchrus* species of greatest taxonomic and nomenclatural interest are wide ranging, all of these studies have bearing on the species to be recognized in Florida and the names they should carry.

The characteristic burs of the *Cenchrus* plant cannot be interpreted easily. The stem and leaves are not special within the grasses. The spikelets (within the bur) are of typical panicoid form, each spikelet with two glumes and two florets, the lower sterile, and each floret enclosed by a lemma and palea. It is the inflorescence that challenges understanding. The entire inflorescence is believed to be a panicle, condensed and reduced to the appearance of a spike. It is further reduced (in most species, not *C. myosuroides*) by lateral branchlets that are much diminished and have coalesced to form indurated spines. These highly modified branchlets enclose one or more spikelets, and form the "bur." In some species, long bristles develop on the lower portions of these spines. In all species, both spines and bristles are retrorsely barbed and non-plumose.

A commentary of the species reported for Florida: *Cenchrus myosuroides* HBK. differs quite markedly from its congeners. It is readily recognized by its many small bur-like heads on a long axis. The spikelets are surrounded by numerous stout bristles fused only at their base. It is by far the most robust of the Florida species, often reaching

1.5 m. or more, in dense growths that exclude competitors. Though tropical, it has a toe-hold on a rocky island in north peninsular Florida off the Levy County coast. It is just as well it is rare, for reports from the Everglades (Wm. Robertson, pers. comm., Dec 1969) that once it gets in one's shirt, the detached prickly bristles persist through two washing and three wearings.

Cenchrus echinatus L. is common, always weedy. Its ruderal habit suggests it may be a recent introduction, but it was often found by early collectors, so must be accepted as native. *Cenchrus brownii* Roem. & Schult., its close ally, is quite rare, presently known from only one or two small, apparently ephemeral populations on the upper Keys. But it is common in the American tropics and has surely washed ashore many times over the years. It also was found by early collectors (e.g. Blodgett, Rugel, on Key West, 1840s), and thus also must be treated as native. Both species are distinguished by the considerable number of flexible bristles on each bur, in addition to the stout spines.

The fearsome *Cenchrus tribuloides* L. is the outstanding species of the entire genus, at least as far as bur size is concerned. [It is surpassed in size, certainly in spine length, by an uncommon species of northwest Mexico, *Cenchrus palmeri* Vasey.] Burs of *C. tribuloides* are the size of grapes, with stout spines extending in all directions. On bur size alone, this species is unmistakable. But its relative rarity and thus unfamiliarity has permitted its name by misunderstanding to be assigned to other, more common species (usually *C. incertus* or *C. pauciflorus*) or to an uncommon tropical species (*C. bambusoides*).

Cenchrus gracillimus Nash, though structurally similar to the others, usually presents no problem in identification. Its narrow leaves are nearly definitive. The Sandhill Sandspur for all practical purposes is a Florida endemic (found only once or twice in southern Georgia). It is almost the only sand-spur to be found in undisturbed "high pine," with wiregrass and other natives. Its Florida range is, in fact, very similar to that of wiregrass (*Aristida stricta*) and longleaf pine (*Pinus palustris*), though these species extend well into Georgia and the Carolinas.

The remaining taxa -- *Cenchrus incertus* M. A. Curtis (1837), *C. pauciflorus* Benth. (1844), *C. longispinus* (Hack.) Fern. (1943; basionym 1903), and *C. bambusoides* Caro & Sanchez (1967) -- represent at least two or perhaps as many as 4 species, with 3 occurring in Florida. Older treatments, both before Chase (1920) and following, usually recognized two species in this group -- *C. incertus* of the eastern United States, and *C. pauciflorus* of the west. Since, limited by early knowledge, only one of these species was to be expected in each region, descriptions tended to be too brief to be diagnostic. Chase (1920; Hitchcock & Chase, 1951), with her wider perspective, believed them to be, but her separation was unclear since she confounded them with *C. longispinus*, a then-poorly recognized species of the midwest and northeast. DeLisle (1963) recognized *C. longispinus* as distinct, but he did not distinguish between the perennial, mostly coastal *C. incertus* and the annual, widespread *C. pauciflorus*. This judgment, uncritically followed, has been the origin of much of the apparently confusing variability found among the sandspurs.

The northern *Cenchrus longispinus* was distinguished by M. L. Fernald (Rhodora 45: 387. 1943) who believed a long-spined, annual plant common in mid-continent could be seen as distinct. This plant is now generally accepted as a species, but appears not to occur in Florida. [Distribution of *C. longispinus* has been mapped by DeLisle (1963, fig. 10). Though some reports are from isolated sites in Florida and it is not unreasonable that this weedy species should have reached the state, all Florida specimens seen bearing this name appear to be misidentified.]

With *Cenchrus longispinus* excluded, taxonomic problems among these related sandspurs are somewhat narrowed but far from resolved. In Argentina, Caro & Sanchez (1967) recognized *Cenchrus incertus* and *C. pauciflorus* (as well as *C. echinatus* and *C. longispinus*). But they also described and named still another species, *Cenchrus bambusoides*, restricted (by their data) to southernmost Florida. Their study relied heavily on foliar anatomy, with clear drawings provided for each species.

Here, *Cenchrus incertus*, *C. pauciflorus*, and, *C. bambusoides*, are all recognized as Florida species. [DeLisle (1963), as noted, recognized only *C. incertus*.]   The position of Shinners, who distinguished *C. incertus* (perennial, decumbent) and *C. pauciflorus* (annual, erect), seems sound, though with enough intermediates to compel caution. Plants corresponding to *C. incertus* are largely coastal, often on dunes, and plants here called *C. pauciflorus* are much more weedy, mostly inland.   But frequently plants are found that seem intermediate.   Either these species produce (apparently) fertile hybrids where they meet, or other distinguishing characters need be found.

But *Cenchrus bambusoides* represents a still higher level of uncertainty.   Caro & Sanchez (1967) gave evidence that this species differed markedly in leaf anatomy.   By their careful drawings, *C. incertus* and *C. pauciflorus* are shown to have rather large bulliform cells just below the upper epidermis, on either side of the blade midline, while *C. bambusoides* has small bulliform cells below the upper epidermis, distributed evenly across the blade. This cellular structure is found throughout the grasses.   As the leaf tissues dry with age or drought, the bulliform cells collapse. Their diminished volume causes distortion of the blade.   In species where the bulliform cells are restricted to near the midline, the flat (=plane) blade folds longitudinally, with the cross-section becoming V-shaped (=conduplicate). And where the bulliform cells are evenly distributed across the width of the blade, drying causes the blade to distort from flat (=plane) to rolled (=inrolled).   Especially in *C. pauciflorus*, a moderately large midvein produces a distinct keel along the ventral surface.

Plants with the foliar characteristics ascribed to *Cenchrus bambusoides* are found in south peninsular Florida, apparently restricted to sandy soils near the shore. But this is also the habitat of *C. incertus* and -- other than the rolled leaves -- Caro & Sanchez's plant is very similar to that species. Also unsettling is the absence of reports of this plant from elsewhere, especially the Caribbean from which have come innumerable other tropical species. The present "best estimate"

(an appropriate statistical term), subject to verification, is that *C. bambusoides* is a recognizable distinct species.

Cenchrus spinifex Cav. (1799), though prior to *C. incertus* and other possible names, is not accepted here. This name was based by A. J. Cavanille on collections from two well-separated South American locations, Longavi [southern Chile] and Montevideo [Uruguay]. Though the description was given in exquisite detail (82 words), it lacks those key phrases that would match it with modern South American species. Chase (1920: 69) rejected the name, stating the leaf blades were not of *C. pauciflorus* and the description and figure of the bur "does not correspond to any known species of *Cenchrus*." DeLisle (1963: 313) stated he had seen a possible isotype (F) that "closely resembles" his all-inclusive *C. incertus*, but rejected Cavanille's name until the isotype label has been verified and the type and isotype have been further studied. Cavanille's name was not recognized or used by Caro & Sanchez (1967). Without comment and without synonymy, Stieber & Wipff (2003: 533) use *C. spinifex* Cav. (perhaps the basis for the appearance of the name in recent floristic publications). However, if DeLisle's tentative acceptance of *C. incertus* were to be reversed, and if Stieber & Wipff's use of *C. spinifex* were to be followed, the meaning of *C. spinifex* would still be subject to uncertainty. As viewed by others, *C. incertus* s.lat. is believed to represent not only *C. incertus* s.str., but a complex of related species, including *C. pauciflorus*, *C. longispinus*, and *C. bambusoides* (even *C. humilis* Hitchc., another South American species). Acceptance of *C. spinifex* would require rejection of the views of Chase (1920), Shinners (1954), DeLisle (1963, tentatively), and Caro & Sanchez (1967), that more than one species is represented by a too-broad interpretation of *C. incertus*. No argument has been seen in support of *C. spinifex*. Certainly acceptance of *C. spinifex* demands a substantial justification.

Without reference to *Cenchrus spinifex*, J. Reveal (Taxon 39: 353-355. 1990) neotypifed *C. carolinianus* Walt. (1788) by material currently known as *C. incertus* M. A. Curtis (1837), thereby displacing that name. His intent was to request the Special Committee (of the Int'l Assoc. for Plant Taxonomy) to declare the name formally rejected (thus

invalid and no longer a threat to *C. incertus*).   But the Committee
declined to act, citing a restriction in the I.C.B.N., thus leaving *C.
carolinianus* the earliest valid name, the very opposite of the goal
sought by Reveal.   Then in 1994, by revision of the I.C.B.N., the
restriction was lifted.   The Committee again addressed the issue, and
the vote was unanimous that *C. carolinianus* be rejected.   Without the
larger world being aware that a nomenclatural catastrophe had been so
narrowly averted, the legitimacy of *C. incertus* was restored.

CENCHRUS L.   Sandspurs [1]

1. Burs small, 1.5-2.5 mm. thick, very numerous (>100) per rachis,
   compactly spaced, forming a long (8-20 cm.) slender spike; bristles
   of bur connate only at base, thus forming no hard involucre (without
   stout spines, the bur scarcely bur-like). Perennial grass, to 2 m.
   Coastal shores. Southwest peninsula (Monroe, Collier cos.);
   disjunct to upper peninsula (Levy Co.); rare (but forbiddingly dense
   where found). Summer-fall.   **Cenchrus myosuroides** HBK.

1. Burs larger, 3-10 mm. thick, few to several (<20) per rachis,
   compactly (in *C. brownii*, *C. pauciflorus*) or loosely spaced,
   forming a stout spike; inner spines of bur connate above base,
   forming a hard involucre around spikelets; inner spines stout (outer
   spines often bristle-like).

   2. Burs large, 6-10 mm. thick; spines hirsute toward base. Annual
      grass, to 0.5 m. Coastal dunes. Florida coastline: panhandle
      (Santa Rosa, Bay, Gulf, Franklin cos.); south peninsula (Palm
      Beach, Collier, Dade, Monroe cos.); northeast peninsula (Duval
      Co.); rare. Summer-fall. The terror of the unshod!
      DUNE SANDSPUR.   **Cenchrus tribuloides** L.

   2. Burs moderate-sized, 3-7 mm. thick (excluding protruding spines);
      spines glabrous or sparsely pubescent.

      3. Bur with many fine bristles encircling base, and a single inner
         whorl of stout flattened spines; plants annual.

         4. Outer spines usually purplish toward tip, 1/2 length of inner
            ones; burs loosely spaced, with rachis apparent; peduncle of
            bur stout, 2.0-2.5 mm. thick. Decumbent annual grass.
            Roadsides, fields, waste areas. Throughout; common. Spring-

summer-fall.
SOUTHERN SANDSPUR. **Cenchrus echinatus** L.

4. Outer spines uniformly tan, ± equal inner ones; burs closely
   crowded, with rachis largely concealed; peduncle of bur
   relatively slender, ±2.0 mm. thick. Annual grass. Waste
   areas, open hammocks, usually near shores. South peninsula
   (Dade, Monroe cos.); rare. Winter-spring. [*Cenchrus viridis*,
   misapplied.] **Cenchrus brownii** Roem. & Schult.

3. Bur with few or no bristles, but with several whorls of stout
   spines, the inner progressively larger; plants perennial or annual.

   5. Leaves narrow, 1.5-3.0 mm. wide; spines very slender, only
      slightly thicker toward base, 3.5-5.5 mm. long. Perennial
      grass. High pinelands, with wiregrass (*Aristida stricta*),
      longleaf pine (*Pinus palustris*). Peninsula (Suwannee, Baker,
      to Dade Co.); frequent. Summer. Nearly endemic.
      SANDHILLS SANDSPUR. **Cenchrus gracillimus** Nash

   5. Leaves broader, 2.5-7.5 mm. wide, spines appreciably thicker
      toward base, 2.0-4.5 mm.long; plants perennial or annual.

      6. Plants annual; leaves plane or folded along a noticeable keel;
         stems somewhat gracile, ascending to erect, usually solitary
         or few; burs closely crowded. Annual grass. Sandy or loamy
         soils of roadsides, lawns, waste areas, sometimes dunes.
         Throughout; common (less so in panhandle). Summer-fall.
         [*Cenchrus incertus*, misapplied.]
         FIELD SANDSPUR. **Cenchrus pauciflorus** Benth.

      6. Plants perennial; leaves plane or inrolled, with a scarcely
         evident keel; stems robust; burs uncrowded.

         7. Burs maturing to dark brown or purple; blades not
            abscising from sheath; stems decumbent, often with several
            from a single base, forming loose mats. Perennial grass.
            Coastal dunes, shelly shores, sandy disturbed soils inland.
            Throughout; common (less so inland). All year.
            [*Cenchrus carolinianus* Walt.; *Cenchrus spinifex*,
            misapplied; *Cenchrus strictus* Chapm.]
            BEACH SANDSPUR. **Cenchrus incertus** M. A. Curtis

         7. Burs tan; blades abscising at summit of sheath; stems one
            or few from a single base, ascending to sub-erect.
            Perennial grass. Sandy shores. South peninsula (Palm

Beach, Collier, Dade, Monroe cos.); infrequent. All year.
                              Cenchrus bambusoides Caro & Sanchez
Excluded names:

  Cenchrus longispinus (Hack. in Kneuck.) Fern.
    Cenchrus pauciflorus, misapplied
    Northern. DeLisle (1963) cited several scattered spms. from
    Florida. But when borrowed and examined (FLAS, 1972) they
    were found to be mostly *C. incertus* in north Florida and *C.*
    bambusoides in south Florida. None, apparently, were true *C.*
    longispinus.

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[1] This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

This study has proceeded by fits and starts over four decades. I am grateful to J. A. Caro, D. G. DeLisle, and L. H. Shinners for their correspondence and willingness to discuss various aspects of *Cenchrus* taxonomy and nomenclature. I thank FSU, ISC, and MO for the loan of specimens to FLAS, and to MEXU for giving me direct access to their collections. David W. Hall, as always, has advised me of problems and possible solutions as he saw them. Scott Forrest, under my direction at FLAS, and Donald R. Deis, under the direction of Daniel F. Austin at FAU, were of significant help on separate but related sandspur projects.

"Jas. C. Neal, M. D." author of the quotation cited above, was recorded as "Entomologist" in the 1889 first annual report of the Florida Agricultural Experiment Station, Lake City, Florida. In the 1890 second annual report he carried the title of "Entomologist and Botanist." He then disappeared from subsequent reports, and from history. Dr. Neal merits commemoration in that he apparently was Florida's first salaried botanist. It wasn't until passage of the Hatch Act in 1887, establishing the nation's agricultural experiment stations, that Florida could support a botanist. Earlier persons with botanical skills were amateur or professional collectors, or supported themselves as physicians or in other ways (see "Botanical Exploration in Florida," by R. P. Wunderlin, B. F. Hansen & J. Beckner, in *Flora of Florida*, vol. 1. 2000).

Erratum - Fossombronia

In the recent paper, *Fossombronia marshii* (Marchantiophyta), a new liverwort species from Arkansas, R. E. Stotler, B. J. Crandall-Stotler and J. R. Bray, Jr. Phytologia 92(2): 230-232 (2010), the terms dioicous and dioecious were inadvertently exchanged. On both line 6 of the abstract (p. 230) and line 11 from the bottom (p. 231) dioecious should read dioicous. We regret this error.

The author (Stotler) points out that dioecious strictly refers to unisexual heteosporous sporophyte plants [vs. monoecious = bisexual] whereas dioicous refers to male and female gametangia on separate gametophytes [vs. monoicous = both male and female gametangia on the same gametophyte]. Flowering plants (and all heterosporous plants) may be either monoecious or dioecious but they are without exception all dioicous.

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(New scientific names and combinations printed in bold face.)

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